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THE PLANT DISEASE REPORTER

Issued By

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 42 **Number** 10

October 15, 1958

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 15 double-spaced typed pages. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

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PLANT DISEASE REPORTER
Mycology and Plant Disease Reporting Section
Crops Protection Research Branch
Plant Industry Station
Beltsville, Maryland

THE PLANT DISEASE REPORTER

Crops Research Division — Plant Industry Station, Beltsville, Maryland
Volume 42 — October 15, 1958 — Number 10

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THE TAXONOMY, ETIOLOGY, AND CONTROL OF COLLETOTRICHUM PIPERATUM (E. & E.) E. & H. AND COLLETOTRICHUM CAPSICI (SYD.) B. & B.[1]

R. W. Smith and D. F. Crossan[2]

Mature sweet-peppers (Capsicum frutescens L.) are parasitized by several melanconiaceous fungi. Colletotrichum piperatum (E. & E.) E. & H. and Colletotrichum capsici (Syd.) B. & B. are generally endemic to pepper growing regions. Based on unpublished work by Smith (5) a third fungus, C. nigrum E. & H., is considered to be synonymous with Gloeosporium piperatum E. & E. The proper combination would be C. piperatum (E. & E.) E. & H. Both C. piperatum and C. capsici are present in Delaware when environmental conditions are conducive to their development. In warm and extremely wet seasons in the Eastern United States, both growers and processors suffer severely through outbreaks of the diseases caused by these organisms.

During the summers of 1956 and 1957, surveys made of contract plantings for the H. P. Cannon & Sons Co., Bridgeville, Delaware, indicated the insidious nature of losses due to anthracnose. In 1956, 3500 mature bell peppers were scored during September for anthracnose-type diseases. Colletotrichum piperatum was found infecting 13.6 percent of these peppers to some degree and, of these, 5.8 percent were classified as cull according to United States Department of Agriculture standards. In 1957, the data obtained from scoring 2000 mature bell pepper fruit during September and October were: 13.4 percent infected to some degree and 3.0 percent cull. Approximately 1500 acres are devoted to production of peppers for processing in Delaware with an average yield of 300 baskets per acre. On the basis of an average weight of 16 pounds per basket, approximately 7,200,000 pounds of peppers are harvested annually. Assuming an average cull loss of 4.4 percent, the loss in pepper fruit due to anthracnose would amount to about 316,800 pounds per year. This represents a potential monetary loss of $9,900.

THE DISEASES

Signs and Symptoms

Colletotrichum piperatum (pepper anthracnose):

Colletotrichum piperatum will attack and cause symptoms on green as well as on red-ripe fruit of a number of pepper varieties (2, 3). The first indication of fungus infection usually appears shortly after the fruit have reddened -- small, indefinite, slightly-sunken, dark yellow spots develop on the surface of the outer flesh. As the fruit nears maturity these spots enlarge and sometimes coalesce. Black acervuli often develop from the darker center of the spot toward the periphery; these acervuli may be arranged in concentric rings, giving a target-like appearance to the spots (Fig. 1).

FIGURE 1. A comparison of the symptoms caused by Colletotrichum capsici and Colletotrichum piperatum on mature fruit of bell-type pepper (Capsicum frutescens L.).

[1] Published with the approval of the Director of the Delaware Agricultural Experiment Station as Miscellaneous Paper No. 312. Contribution No. 108 of the Department of Plant Pathology.

[2] Graduate Research Assistant and Assistant Research Professor, respectively, Department of Plant Pathology, University of Delaware.

After invading the outer hull of the fruit, the fungus grows into the seed cavity and infects the seed. Lightly infected seed may appear normal, but severely infected seed are dark in appearance.

The fungus was isolated from small, tan flecks appearing on cotyledonary leaves shortly after they had expanded from the seed. It is presumed that the fungus is transferred to stems and petioles of the plant from the lesions on the cotyledonary leaves by spashing rain. Leaf symptoms were found to develop only rarely even under severe disease conditions. Dieback of stems has been observed on certain occasions when spore suspensions were applied to certain "hot varieties" of pepper.

Colletotrichum capsici (ripe rot):

C. capsici also causes disease symptoms on both green and red pepper fruit (6). Generally, the initial symptoms are similar to those caused by C. piperatum; however, setae usually develop and give a dark appearance to an infected area (Fig. 1). Like C. piperatum, C. capsici also advances into the seed cavity and infects as well as infests the seed.

Using artifical inoculation techniques on some 40 varieties of pepper under greenhouse conditions, no real differences were discernible in type symptoms found on the fruit. All symptoms generally corresponded to the description given in the section for C. piperatum.

Etiology

Overwintering of C. piperatum:

Conclusive experimental evidence concerning survival of C. piperatum could not be found in the literature. Higgins (3) believed the main means of survival of the fungus was via seed-borne spores, such spores being transferred to, and causing infection on, the primary leaves as they expanded from the seed. In the present investigation, tests were performed to determine experimentally (a) if the C. piperatum fungus could overwinter under Delaware field conditions, (b) in what form the fungus overwintered, (c) how long the fungus could survive on infested seed, and (d) in what form the fungus survived in or on seed.

To obtain data on the overwintering of C. piperatum, infected fruit collected in September 1956 were incorporated into the soil in two isolated plots. In the spring of 1957, 15 disease-free plants grown from mercuric chloride-treated seed were planted in each of the two plots. An additional two plots which had not received disease refuse were planted at the same time. The plots were watered daily with a forceful stream of water to allow the soil to splash on the various parts of the plants. Data were obtained on the percentage of anthracnose-infected mature fruit harvested from plots with and without diseased refuse. Results obtained are presented in Table 1.

Table 1. Number of anthracnose-infected and healthy fruit harvested from plots with and without disease refuse.[a]

Plot	Rep. 1	Rep. 2	Total infected fruit
Infested	6	14	20
Non-infested	0	1	1

[a] 15 plants were scored in each plot, with a total of three pickings during the season. Malathion insecticide applied once during growing season.

The one infected fruit in the non-infested plot was thought to have occurred because of transfer of inoculum by several wind-driven rains just prior to the last picking.

To determine the form or stage in which the fungus survived, soil samples were taken from the infested plots during February of 1957 and screened to obtain plant debris. Samples of the refuse were surface-sterilized and cultured on artificial media to determine whether C. piperatum were present.

Microscopic examination of the refuse revealed only asexual structures of the fungus in the form of stromatic bodies containing embedded conidia. This stroma-conidial-containing

material was identified, after incubation on acidified PDA, as C. piperatum. Artificial inoculations with cultures originating from these stroma resulted in typical anthracnose symptoms on pepper fruits.

Survival of C. piperatum and C. capsici on infected seed:

Laboratory experiments were conducted to determine longevity of C. piperatum on dry seed harvested from artificially infected fruit. Viability of C. capsici also was determined in a similar experiment, since experimental evidence was lacking on this aspect of the life cycle of this fungus.

Seeds were harvested from fruits artificially inoculated with the two fungi, dried, and placed in glass containers. Seeds from each container were planted, five to a plate, at monthly intervals on pepper-decoction agar in 90 mm Petri plates. Presence or absence of fungus growth was recorded after 1 week of incubation at 28° C. Final identification of the two fungi was made when sporulation occurred. The experiment was carried over a 9-month period. Results of the experiment are shown in Table 2.

Table 2. Survival of C. capsici and C. piperatum on pepper seed infected March 11, 1957.

Seeding date	Number of seeds	Number of seeds exhibiting fungus growth[a]	
		C. capsici	C. piperatum
April 9	50	23	20
May 10	50	22	20
June 11	50	23	18
July 16	50	20	20
August 20	50	20	15
September 27	50	21	17
October 31	50	23	15
November 31	25	13	10
December 31	25	12	9

[a] The data indicate that both C. capsici and C. piperatum are capable of surviving in or on dry pepper seed for at least 9 months.

Microscopic examinations and simple staining techniques were employed to determine the form in which the two fungi survived on the seed. Cleared, free-hand sections of seeds were stained with a lacto-phenol-cotton-blue stain. Acervuli could be clearly distinguished on the outside of the seedcoat of seeds infected with either of the two fungi. Strands of hyphae were found ramifying various portions of the internal seed tissues. When seeds infected with either of the two fungi were immersed in a drop of water and the water examined, a multitude of spores could be observed; however, these spores were found to be non-viable in the 9-months old seed sample.

CONTROL

Fungicides

Two separate field tests were conducted at Newark, Delaware during the summer of 1957 to determine the effectiveness of certain commercially available fungicides in controlling "pepper anthracnose disease" (Colletotrichum piperatum), and "ripe rot disease" (Colletotrichum capsici).

Artificial inoculation techniques were used at two University Farm field sites. The fungicides zineb, maneb, and captan were screened in both the field tests; Tri-basic copper sulfate was also screened at the "anthracnose disease" control plot.

A randomized, replicated block design was used in the field plots, consisting of treatment rows alternated with buffer rows at 3 1/2 foot spacing. Each treatment was replicated four

times. Fifteen plants of the selection Caldel were planted at a 2 1/2 foot spacing in all rows.

Spore suspensions of C. piperatum and C. capsici were used as inoculum for the plants on the two isolated plots. Inoculum was prepared by growing the fungi on Petri plates containing pepper-decoction agar. Their contents were macerated separately in a Waring Blendor with a small amount of tap water. The spore and mycelial suspensions were then diluted with 3 gallons of additional water, poured into 3 1/2 gallon John Bean knapsack sprayers, and separately sprayed on the treatment rows. Fungicides were applied in the afternoon of a given day and the inoculum was applied approximately 6 hours later during the evening of the same day. The fungicide sprays and inoculum were first applied approximately 4 weeks after blossoming of the peppers and were repeated three times at 2-week intervals.

Mature red fruit were harvested from September 9 to October 7 and the following records taken: (a) number of anthracnose infected fruit; (b) number of marketable fruit; and (c) weight of marketable fruit.

The data obtained indicated that, with a high incidence of either disease, three applications of zineb or maneb fungicides applied at 14-day intervals, beginning approximately 4 weeks after blossoming, resulted in a significant reduction in the number of diseased fruit (Tables 3, 4). The fungicide-treated plants showed significantly greater yields in weight of marketable fruit. Data concerning the control of ripe rot disease are not in agreement with recent data presented by Chandler (1) in Georgia. In his tests Chandler did not obtain significant control of "ripe rot disease" with any of 17 materials screened. Among these materials were those presented in this paper.

Table 3. Number of C. capsici-infected fruit harvested from fungicide plots at Newark, Delaware, September 1957 - October 1957.

Treatment[a]	Concentration	Total fruit picked	Mean number infected fruit[b]	Percent infected fruit
Check	--	126	29.0	92.0
Captan	2-100	160	20.0	51.9
Zineb	2-100	196	8.8	17.3
Maneb	2-100	179	8.5	19.6
LSD at 5% level			8.8	
LSD at 1% level			12.7	

[a] First application approximately 4 weeks after blossoming and repeated at 14-day intervals for a total of three applications.
[b] Plants inoculated after each fungicidal application.

Table 4. Number of C. piperatum-infected fruit harvested from fungicide plots at Newark, Delaware, September 1957 - October 1957.

Treatment[a]	Concentration	Total fruit picked	Mean number infected fruit[b]	Percent infected fruit
Check	--	183	33.0	71.4
Tri-basic copper sulfate	2-100	210	21.4	40.6
Captan	2-100	233	13.7	23.53
Zineb	2-100	250	12.0	17.85
Maneb	2-100	269	8.7	13.92
LSD at 5% level			7.3	
LSD at 1% level			13.1	

[a] First application approximately 4 weeks after blossoming and repeated at 14-day intervals for a total of three applications.
[b] Plants inoculated after each fungicidal application.

Host Susceptibility or Resistance

Experimental evidence concerning host susceptibility or resistance to the C. piperatum and C. capsici fungi is negligible. Malabanan (4) states that the sweet pepper varieties of the fleshy Ruby King type are susceptible to C. nigrum, whereas hot varieties are very resistant.

In the present investigation, screening tests for host susceptibility were made in both the greenhouse and the field. The plants were artificially inoculated with spore suspensions of the respective fungi.

All of the following varieties or selections were susceptible to both C. piperatum and C. capsici: Sunnybrook, Yolo Wonder, Ruby Giant, Caldel, Florida Giant, Pyramid Wax, Sweet Banana, California Wonder, Vinedale, Ruby King, Cuban, Chinese Giant, King of the North, Bullnose, Early California Wonder, Truhart Pimento, Keystone Wonder Giant, All Big, Improved King, Rocky Ford, Oakview Wonder, Hungarian Yellow Wax, and Anaheim Chilli; Red Cherry was susceptible to C. capsici but not to C. piperatum. Cascabella, Mexican Chilli and Italian El were moderately resistant to both C. piperatum and C. capsici.

CONCLUSIONS

Anthracnose of pepper, caused by Colletotrichum piperatum (E. & E.) E. & H., causes insidious but meaningful losses in pepper fields in Delaware. Losses in excess of 50 percent of marketable fruit have been reported but more often losses are less than 5 percent.

C. piperatum can survive in refuse in or on the soil surface in the form of stromatic bodies containing imbedded conidia for at least 9 months under Delaware conditions; furthermore, the surviving fungus is capable of contaminating and causing infection on pepper seedlings and fruit. C. piperatum and C. capsici were found capable of surviving in or on dry pepper seed for at least 9 months. Acervuli could clearly be distinguished on the outside of the seed coats, and strands of hyphae were found ramifying various portions of the internal seed tissues. Conidia were found in abundance clinging to the seed coat; however, these were found to be non-viable.

Certain commercial fungicides were found to be effective in reducing the fruit rots caused by C. piperatum and C. capsici. Under high disease incidence, zineb or maneb applied at 14-day intervals, beginning approximately 4 weeks after the peppers had bloomed, reduced significantly the amount of disease and gave greater marketable yields.

In screening for susceptibility and/or resistance to C. piperatum and C. capsici, only one sweet, tapering variety, Italian El, was found moderately resistant to both diseases. Several "hot" varieties seemed to exhibit resistance to both fungi (Cascabella and Mexican Chilli).

Fruit symptoms caused by the two pathogens are quite similar. However, the causal organisms are distinguishable on the basis of the extreme setae-bearing characteristic of the ripe-rot fungus. C. capsici is further distinguished by its falcate spores. Both fungi have been noted attacking the stems of pepper plants as well as the seed. The anthracnose fungus has also been isolated from leaf and cotyledonary lesions.

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CLADOSPORIUM ON MELONS IN SOUTH TEXAS

Bailey Sleeth

Abstract

Cladosporium cucumerinum Ell. & Arth. was found on leaves, petioles and stems of crown blighted watermelons and cantaloupes in South Texas, early in the growing season of 1958. The fungus was found to have infected the nets of apparently healthy cantaloupes in the field. Net infection was observed on 35 selections of cantaloupes in a variety and selection trial. The intensity of net infection increased with the maturity of the cantaloupe fruits.

A crown blight of both watermelons and cantaloupes was common during the early part of the 1958 growing season in the Lower Rio Grande Valley and nearby melon producing areas in South Texas. Symptoms, lesions and necrotic areas on the foliage were similar to those described for scab and caused by Cladosporium cucumerinum Ell. & Arth. On the crown leaves symptoms appeared early in the growing season and developed rapidly in a short time, after which spread of the disease slowed down and apparently stopped. This rapid and abrupt decline in infection and spread of the disease on the leaves was associated with a change from cool to hot weather.

Cladosporium was found to be fruiting abundantly on necrotic areas of watermelon leaves, petioles and stems, May 6, 1958, on affected plants brought to the laboratory from the Falfurrias area. The grower had suspected downy mildew, but no mildew symptoms were found. When the melon fields were inspected a few days later, crown blight was found to be prevalent in most of the fields. Infection appeared to be confined to the crown leaves and not actively spreading. Necrotic areas had developed as leaf spots and on the margins of the leaves. Often a few of the oldest leaves of a plant would be dead. Lesions on the petioles and especially on the stems were found at considerable distances from the crown-blighted center and beneath leaves with no dead spots. Stem lesions were found frequently to occur within a short distance of the growing tip of the vines.

Crown blighted cantaloupe plants were found to be infected with Cladosporium at Weslaco in the Lower Rio Grande Valley. As in the case of watermelons, the fungus was found to be fruiting abundantly on necrotic lesions on the leaves, petioles and stems. In addition to foliage infection the fungus was found well distributed on the nets of healthy-appearing cantaloupes. In mature melons, full slip, typical fruiting developed in abundance on the margins of the net when kept in a cool moist chamber for 12 hours, or over night. Also, sporulation occasionally occurred on the nets when cantaloupes were left over night in the laboratory, even though not kept in a moist chamber.

Cladosporium net infection was found in 35 varieties or selections of cantaloupes growing in a Station variety trial planting. Intensity of net infection increased with the maturity of the melons. No effort was made to determine when net infection might occur; however, melons too green to pick for marketing were found to be infected.

C. cucumerinum was readily isolated from necrotic lesions on the leaves, petioles, stems, and the net of cantaloupe fruits. No pathogenicity tests were run on growing plants. Attempts to cause rind rot of cantaloupes were not successful. Negative results may possibly have been the result of the high temperatures that existed in the laboratory.

The finding of Cladosporium cucumerinum in association with crown blight of watermelons and cantaloupes and fruiting on the nets of cantaloupes suggests that this fungus may be an important factor in the crown blight complex in the Lower Rio Grande Valley and South Texas. Other fungi, such as Alternaria, which was frequently found on diseased leaves, are apparently a part of the crown blight complex. Also, these fungi, especially Cladosporium which infects the net of cantaloupes in the field, undoubtedly cause rotting of cantaloupes in transit and in the markets.

TEXAS AGRICULTURAL EXPERIMENT STATION, SUBSTATION NUMBER 15, WESLACO, TEXAS

MYCELIAL SEED INFECTION OF CERTAIN CRUCIFERS BY SCLEROTINIA SCLEROTIORUM (LIB.) D BY.

Paul Neergaard[1]

Summary

In connection with routine seed health testing in Denmark mycelial seed infection by Sclerotinia sclerotiorum has been observed in 23 out of a total of 1045 Danish seed lots of Brassica oleracea (22 of 801 lots), Brassica napus var. napobrassica (1 of 174 lots) and Brassica campestris (0 of 74 lots). The probable importance of dead flower parts as source of mycelial infection of seeds is emphasized.

INTRODUCTION

Recently D. M. McLean (2) demonstrated the importance of dead flower parts in infection of leaves of certain crucifers by Sclerotinia sclerotiorum. This interesting observation suggests as an entailing assumption the equal importance of dead flower parts in promoting mycelial infections in young seed primordia and maturing seeds.

Seed infestation or infection by Sclerotinia sclerotiorum in crucifers has been reported in several cases, partly as a) sclerotia accompanying the seed (1), partly, and this is of particular interest in connection with the discussed relationship, as b) mycelial infection of the seeds (7).

MATERIAL AND METHODS

During the last 5 years observations on seed infection of the last mentioned category have been made during the routine seed testing for seed-borne diseases on crucifers (particularly for Alternaria brassicae, Alternaria brassicicola and Phoma lingam) at the Government Plant Protection Service, Copenhagen (3, 4, 5, 6). About a thousand commercial seed lots have been tested in the period mentioned at the request of seed firms applying for seed health certificates.

The following technique is used: At least 1000 seeds of each seed lot are sown in Petri dishes on blotter paper moistened with a 0.2 percent solution of 2,4-bichlorophenoxyacetate in order to prevent germination of the seeds. The seeds are kept at room temperature and examined after 5 and 9 to 10 days.

RESULTS

The numbers of infected seed lots and the total numbers of seed lots investigated are given in Table 1. The years indicated are the winter seasons during which the seeds were examined. Practically all of the seed lots investigated are harvested in the preceding year.

In none of the infected seed lots in question have sclerotia been observed. In many cases sclerotia are removed in the ordinary milling operation, being collected in the screenings in great amounts (1), and leaving among the seeds only sclerotia of about the same size and shape as the seeds.

Using the technique described above, infection by Sclerotinia sclerotiorum usually has been observed at the first examination (5 days after sowing) as white mycelial growth spreading from one seed and infecting the surrounding seeds, causing a soft seed rot. By the second examination (after 10 days) profuse mycelial growth, especially in the periphery of the colony of the fungus, can be seen as well as a vivid development of sclerotia.

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Table 1. Mycelial seed infection by Sclerotinia sclerotiorum in Danish seed lots of Brassica oleracea, Brassica napus var. napobrassica and Brassica campestris, 1953-1958. In parentheses, total numbers of seed lots investigated.

Variety	1953-1954		1954-1955		1955-1956		1956-1957		1957-1958		Total 1953-1958	
Cabbage		(98)	1	(98)	4	(91)	6	(81)		(73)	11	(441)
Red cabbage		(32)		(24)		(19)		(15)		(17)	0	(107)
Savoy cabbage		(18)		(25)		(25)	1	(31)		(28)	1	(127)
Spring cabbage	1	(18)		(8)		(8)	1	(11)		(15)	2	(60)
Brussels sprouts		(10)		(14)		(9)		(6)		(20)	0	(59)
Cauliflower		(97)	1	(74)	2	(75)	1	(77)		(64)	4	(387)
Kale		(14)		(3)	1	(9)	1	(15)		(11)	2	(42)
Swede	1	(47)		(40)		(31)		(28)		(24)	1	(170)
Turnip		(29)		(10)		(6)		(16)		(13)	0	(74)
Other Brassica species		(4)		(48)	1	(11)		(14)	1	(11)	2	(88)
Total											23	(1045)

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EFFECTS OF FUNGICIDE COMBINATIONS ON CONTROL OF CELERY EARLY BLIGHT

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Summary

Dyrene (2,4 dichloro-6-(o-chloroanilino)-s-triazine) plus Tribasic Copper Sulfate, and Dyrene plus Parzate (zineb; wettable powder) were additive in their effects on the control of celery early blight (Cercospora apii Fres.). Tank mix zineb (nabam plus zinc sulfate) gave significantly better control of the disease than did pre-prepared zineb (Parzate; wettable powder) at an equivalent concentration.

INTRODUCTION

Celery seedbeds in the Everglades area are sown as early as June and sowing continues through October. Harvesting of celery from the field is continuous from October to June. Therefore, since celery is a perennial crop in the area, early blight (Cercospora apii Fres.) becomes a perennial problem. Its severity at times necessitates the abandonment of large acreages due to lack of adequate control. Combinations of materials with synergistic effects would substantially increase disease control when maximum severity exists as a result of optimum disease conditions.

Synergism between Parzate (zineb) and Thylate (thiram) for control of celery early blight and anthracnose (Colletotrichum sp.) has been suggested by Cox (1, 3). The possibility of synergism between Dyrene and Tribasic Copper Sulfate is suggested also, based on past performances for control of early blight. The following experiment tests the hypothesis for synergism.

METHOD AND MATERIALS

Three-month-old plants of the Utah 52-70 variety were set with the aid of a power celery planter in 50-foot rows, 100 plants per row. The rows were 36 inches apart. Each treatment was replicated four times in a randomized block design with guard rows between each treatment.

Plants were set out October 8 after appropriate fertilizer application. General application of DDT and parathion for aphid and leaf miner control was maintained weekly.

Spraying was done with the aid of a power sprayer, described elsewhere (2), at 300 p.s.i. Spray applications ranged from 56 to 170 gallons per acre, depending upon size of the plants. A 3- to 6-day spray schedule was maintained, with a total of 14 applications. Disease severity ratings were taken after 12 and 14 applications.

The results of periodic random isolations of foliar lesions present in a supplemental check row showed that early blight was the only foliar disease present. Rhizoctonia petiole rot was not observed in the plantation.

RESULTS

Prior to November 22 no early blight was present in the plantation; however, on November 22 it appeared sporadically and was of minimum intensity. Within five days the disease was of severe intensity throughout the plantation.

No toxicity in the form of foliar discoloration was observed as a result of any spray treatment. On the one extreme Acti-dione caused stunting; on the other the nabam plus zinc sulfate treatment resulted in taller, fuller foliaged plants. Yield data were not obtained because of inclement weather.

The following results are based on data from Table 1 when the disease severity ratings were obtained on December 9, 1957 and the L.S.D was at the 5 percent level.

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Table 1. Effects of single and combination fungicides on the control of celery early blight, Belle Glade, Florida, 1957.

Treatment[a]	Concentration	Disease severity[b] Dec. 2	Dec. 9	Group
1. Nabam-$ZnSO_4$	2 qt.-3/4 lb.	0.4	1.8	
2. Dyrene-TBCS	1 1/2 lb.-3 lb.	0.8	2.0	
3. Dyrene-Parzate	1 lb.-1 lb.	1.3	2.2	A
4. Dyrene	2 lb.	1.3	2.9	
5. Dyrene-Thylate	1 lb.-1 lb.	2.3	2.9	
6. Dyrene	1 1/2 lb.	1.9	3.3	
7. Parzate-Thylate	2 lb.-1 lb.	1.4	3.3	
8. Parzate-Thylate	1 lb.-1 lb.	2.8	3.5	
9. Manzate	2 lb.	3.1	3.9	B
10. Parzate	1 lb.	3.4	3.9	
11. Dyrene	1 lb.	2.9	4.0	
12. Parzate	2 lb.	3.6	4.0	
13. Manzate	1 lb.	5.0	4.1	
14. Manzate-Thylate	1 lb.-1 lb.	3.3	4.1	
15. TBCS	3 lb.	5.4	4.6	
16. Thylate	2 lb.	5.5	5.4	C
17. Thylate	1 lb.	6.4	5.4	
18. Acti-dione O	25 ppm	7.0	7.1	
19. Agri-mycin 100	100 ppm	6.9	7.3	D
20. Control (Water)	-	7.9	8.1	
L.S.D. 5%		1.40	1.13	

[a] Nabam: 19% (disodium ethylene bis-dithiocarbamate).
Dyrene: 50% 2,4-dichloro-6-(o-chloroanilino)-s-triazine.
Parzate: 65% zineb (zinc ethylene bis-dithiocarbamate).
Thylate: 65% thiram (tetramethylthiuram disulfide).
Manzate: 70% maneb (manganese ethylene bis-dithiocarbamate).
TBCS: 53% copper in Tri-basic Copper Sulfate ($CuSO_4 \cdot 3Cu(OH)_2$).
Acti-dione O: 1% oxime of cycloheximide.
Agri-mycin 100: streptomycin 15%, oxytetracycline 1.5%.

[b] Disease severity based on 0 (no disease) to 11 (maximum severity).

I. Comparative Control

Group A: Nabam plus zinc sulfate was rated as the best control; however, it was statistically identical with others in Group A.

Group B:

1. Parzate 1 pound plus Thylate 1 pound was rated better than Manzate 2 pounds, Parzate 2 pounds, and Manzate 1 pound plus Thylate 1 pound; however, none of these treatments was significantly different.

2. Parzate 1 pound plus Thylate 1 pound, Manzate 2 pounds, and Parzate 2 pounds were not significantly different from Dyrene 2 pounds or Dyrene 1 pound plus Thylate 1 pound (both in Group A). Manzate 1 pound plus Thylate 1 pound, however, was significantly different from the latter two treatments.

Group C:

1. TBCS 3 pounds had a lower disease control rating than either Thylate 2 pounds or Thylate 1 pound. Doubling the concentration of Thylate did not decrease the disease severity rating.

2. TBCS 3 pounds did not give significantly different results from those obtained from Parzate 1 pound plus Thylate 1 pound, Manzate 2 pounds, Parzate 2 pounds, or Manzate 1 pound plus Thylate 1 pound.

Table 2. Comparison of the reciprocals of single and combination fungicide ratings for control of celery early blight.

Combination		Single ratings $(\frac{1}{R})$*		Sum of single ratings	Combination ratings $(\frac{1}{R})$*	Effect
A	B	A	B	A + B	AB	
Dyrene	- TBCS	0.30	0.22	0.52	0.50	Additive
Dyrene	- Parzate	0.25	0.26	0.51	0.45	Additive
Dyrene	- Thylate	0.25	0.19	0.44	0.34	Non-additive
Parzate	- Thylate	0.26	0.19	0.45	0.29	Non-additive
Manzate	- Thylate	0.24	0.19	0.43	0.24	Non-additive

* "R" is the rating shown in Table 1 when the ratings were taken on December 9, 1957.

Group D: Acti-dione O at 25 ppm and Agri-mycin 100 at 100 ppm were not significantly different from the untreated control. The ratings within Group D were significantly different from any other group.

II. Fungicide Combination Effects

Five combinations of fungicides were tested within the scope of the experiment for synergistic effects on control of early blight. The results, based on the statistical differentiation in Table 1, are as follows:

1. Dyrene 1 1/2 pound plus TBCS 3 pounds was significantly different from Dyrene 1 1/2 pound, both as an individual treatment and on a group basis.
2. Dyrene 1 pound and Parzate 1 pound had significantly the same rating, whereas Dyrene 1 pound plus Parzate 1 pound had a significantly higher rating than the single-fungicide treatments.
3. Dyrene 1 pound plus Thylate 1 pound was not significantly different from the Dyrene 1 pound rating.
4. The rating of Parzate 1 pound plus Thylate 1 pound was not significantly different from that of Parzate 1 pound.
5. Manzate 1 pound plus Thylate 1 pound was the same as Manzate 1 pound.

Table 2 compares the effects of combination treatments on a numerical basis when "synergism" is defined as "the total effect being greater than the sum of the two effects taken independently" and "addition" as "the total effect being equal to the sum of the two effects taken independently."

CONCLUSIONS

(a). Nabam plus zinc sulfate (zineb) gives significantly better control of celery early blight than does Parzate (zineb; wettable powder) at an equivalent concentration.

(b). Doubling the concentration of thiram has no effect on control of the disease.

(c). Dyrene plus TBCS and Dyrene plus Parzate are additive in their effects for control of early blight.

(d). Dyrene plus Thylate and Manzate plus Thylate are not additive.

(e). The hypothesis for synergism is rejected.

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VERTICILLIUM WILT OF CICER ARIETINUM IN SOUTHERN CALIFORNIA

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Since 1954 in the San Pedro hills of Los Angeles County, California, a wilt disease of garbanzo bean plants (Cicer arietinum L.) incited by Verticillium albo-atrum Reinke & Berth. has occasionally been seen. Foliage of affected plants was yellow in color and eventually plants wilted and died. A light brown discoloration of the xylem tissue was also present in infected plants.

When young 2-week-old seedlings grown in steamed soil were inoculated by dipping the roots in a spore and mycelial suspension of Verticillium, isolated from diseased stem tissue, 47 percent of the plants wilted and showed xylem discoloration. A summary of the results of four experiments is presented in Table 1. Reisolation of Verticillium from the basal part of the stems was successful.

No infection, as indicated by symptom expression or re-isolation of the fungus, was detected in one experiment in which undisturbed plants were grown in artificially infested soil. This may indicate that some injury is necessary for natural infection to occur.

These data indicate that garbanzo bean is a host of Verticillium albo-atrum. No other report of the pathogenicity of this fungus on garbanzo bean has been seen. An abstract of this work has been published[1] previously.

Table 1. Effect of inoculation of Cicer arietinum plants by dipping roots into a spore suspension of Verticillium albo-atrum.

Treatment	Total number plants	Number plants dead	Percent plants (including dead plants) with xylem discoloration
Inoculated[a]	92	14	47
Check	74	1[b]	0

[a]Inoculation data represent a summary of four experiments.
[b]Plant was affected by a root rot of undetermined cause.

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SOME FACTORS IN THE CONTROL OF BLUE-GREEN MOLD DECAY OF CITRUS FRUIT WITH AMMONIA

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Summary

Under certain conditions ammonia released in fiberboard cartons of inoculated oranges will protect the fruit from decay caused by Penicillium italicum Wehmer and P. digitatum Sacc. The purpose of the investigations reported here was to determine at which time or times following inoculation a quantity of gas noninjurious to the fruit was fungicidally most effective. This was accomplished by injecting various quantities of the gas into cartons of fruit on one or more of several days following inoculation. Injections of 1/8, 1/4, 1/2, or 3/4 grams of anhydrous gaseous ammonia per carton were made in several combinations on the first, fifth, and ninth days. Those made on the first day were less effective than those made later. The best control of decay was effected by two or three injections of 1/2 or 3/4 grams of ammonia. Following injection experiments, the fungicidal effectiveness of various patterns of ammonia released from chemicals was studied under the moist conditions found in cartons of inoculated oranges. The dried chemicals in pellet form or on paper sheets were placed in the cartons and then removed at various times following inoculation and analyzed for the amount of ammonium compound remaining. From these results the amounts of ammonia released during the several periods were determined and related to the percentage of decay control. Under simulated refrigerator car conditions decay control was proportional to the amount of ammonia released. The chemicals emitted much less ammonia and were less effective at 32° than at 50° or 68° F.

INTRODUCTION

The blue-green mold decay caused by Penicillium italicum Wehmer and P. digitatum Sacc. is today the most important post-harvest decay of citrus fruit (10, 11). The conversion in recent years from individually wrapped fruit packed in wooden boxes to unwrapped fruit in fiberboard cartons has accentuated the decay problem, and the need for a volatile chemical in the packed container to protect fruit from decay has now become a necessity. The chemical diphenyl, a volatile fungistat, has been used for this purpose. It was first screened by Tomkins (17) and was developed for commercial use by Farkas (3).

Diphenyl is normally placed in each carton on two sheets of paper measuring 11 x 18 inches or in the paper tissue used for wrapping individual fruit. Its effectiveness as a fungistat for citrus pathogens has been demonstrated by many workers (3, 8, 12).

Since 1953 an intensive screening program[2] has been under way to find a substitute treatment for diphenyl as an in-package protectant of citrus which would not have the undesirable characteristics of residue, odor, or off-flavor attributed to diphenyl. Among the many compounds screened, the ammonia-emitting materials were consistently promising for controlling Penicillium decay. Ammonium bicarbonate was first used as a citrus preservative in 1926 by Bottini (2). It was subsequently tested in England and in Africa (1, 4, 18) and more recently in Florida (9). Though control of decay in all tests was achieved it was reported to cause burn-like injury to the fruit surface (1, 4, 9). Recent studies have shown that ammonia gas can be used to control decay without causing injury (7, 13, 15, 16). Gunther and co-workers (5, 7) synthe-

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[2] Acknowledgment is made for fruit and funds supplied by Sunkist Growers, Inc., Pure Gold, and American National Foods, Inc. To Mr. J. R. MacRill, Manager of the Sunkist Field Laboratory, grateful acknowledgment is made for procuring and compartmenting the two refrigerator cars used in the experiments. Thanks are extended to the Santa Fe Railroad Company for the loan of the cars.

sized many ammonia-releasing compounds and formulated them as loose powders, tablets, or powders between two sheets of paper one of which is porous and the other made impervious with a polyethylene coating. These formulations provided a more uniform release and distribution of gaseous ammonia than did ammonium carbonate. The prepared formulations were extensively tested for control of the blue-green decay, and some of the more effective formulations were prepared commercially.

In attempting to formulate ammonia-emitting compounds for use in fiberboard cartons, the quantities of gaseous ammonia which prevented decay when injected at various times into cartons of injured and inoculated fruit were first determined. After the optimum range of concentration and time of application for best decay control were learned, a study was made to determine ammonia release patterns from the ammonia-emitting compound that most nearly approached the optimum indicated by the gas studies. Most of these tests were conducted under simulated refrigerator car conditions.

MATERIALS AND METHODS

Fruit Inoculation

The rind of oranges or lemons was wounded by cutting a wound approximately 1 mm wide, 1 mm deep, and 10 to 15 mm long with a circular saw encased in a plastic housing as previously described (14); however, in this work, no liquid spore suspension was used in the plastic housing. Instead, the wounded fruits were inoculated by placing them with the wounded side up in a chamber holding approximately 150 fruits (Fig. 1) and showering them uniformly with 1/2 or 1 cubic cm of dry Penicillium spores. To accomplish this, spores were injected into the chamber in the draft of a revolving fan for 1 minute and the spores allowed to settle for 4 minutes before the front of the chamber was opened and the fruit removed. Spore counts made from vaselined slides placed at various locations on top of the fruit have consistently shown a uniform distribution of spores. A shower with 1 cubic cm of dry spores yielded approximately 4000 spores per square cm.

Gas Injection Studies

For the gas injection studies navel oranges were wounded and showered with dry Penicillium spores as described. The fruits were then randomly sorted into telescope-type cartons 10 x 11 x 16 1/2 inches, vented at both the top and bottom with two 1 x 4-inch slots. Eighty-one cartons of fruit were arranged in stacks three high in one section of a four-compartmented iced railroad car. Three cartons each of diphenyl-treated and untreated check fruits were held at separate ends of a second compartment. An overhead fan which operated intermittently, remaining off for a total of 7 hours daily, approximated the stop and go periods during transit across country. The conditions which could not be duplicated for an exactly simulated shipment were the vibration effects of a car in motion and a full load of fruit and cartons in each compartment.

Ammonia gas was injected into each carton through holes cut in the bottom center of the carton. The quantity of gas used for each injection was metered through a flow-meter over a 30-second interval. Twenty-eight different combinations of concentration and time of application were applied. Ammonia concentrations of 1/8, 1/4, 1/2, and 3/4 grams per carton, respectively, were injected on the 1st; 5th; 9th; 1st and 5th; 1st and 9th; 5th and 9th; and 1st, 5th, and 9th days after inoculation. There were three replications for each treatment.

Ammonia Emission Patterns

Packets of tablets (called Winn-Mats) used for these studies are produced commercially by the Winning-Peplow Company, Los Angeles, California; they are shown in Figure 2. These tablet mats consist of nine tablets 1/8 inch thick and 3/4 inch in diameter which are sandwiched between a polyethylene-coated paper sheet and a white porous absorbent sheet. The mats were placed in the center of each carton with the white absorbent side downward. The tablets consist of a mixture of ammonium sulfate, soda ash, and various fillers (5, 7). They release ammonia by chemical reaction initiated by the absorption of moisture transpired from the fruit. Eighteen tablets are used per standard carton and contain an average of 2.1 gm of available ammonia. The ammonia emission patterns determined in these studies are represented by graphs of the release of ammonia gas in relation to time from formulations in the presence of fruits during a

FIGURE 1. Chamber used for inoculation of citrus fruit with Penicillium spores; 1 cubic cm of dry spores is placed in glass measuring tube (shown above), injected into chamber in the draft of revolving fan by squeezing deVilbiss bulb, and allowed to settle on wounded fruit (wound facing up).

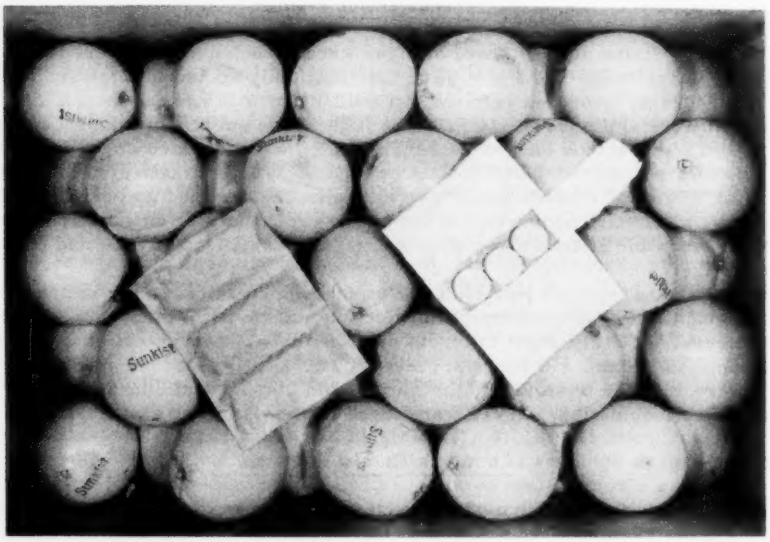

FIGURE 2. Packaged tablets placed in a carton of fruit. A section of the absorbent side is cut away to show the tablets. Two mats are used per carton and each mat holds nine tablets. The total ammonia per two mats averages 2.1 gm.

normal shipping period of about 14 days. They are obtained by removing the tablets or sheets periodically from the cartons of fruit and analyzing them for their ammonia content. The average of replicates of ammonia remaining in the mats is subtracted from the ammonia content of a representative sister lot of unused pellets or sheets. The differences represent the ammonia emitted during the several time intervals and, when graphed, represent the rate pattern of ammonia release.

Ammonia-emitting sheets used in the constant temperature studies consisted of two separate pieces of absorbent paper towelling, each sheet measuring 11 x 17 inches. The sheets were prepared by the Crown-Zellerbach Corporation; one, impregnated with ammonium sulfate solution and dried; the other, saturated with sodium carbonate solution and dried. A set consisting of one of each type of sheet was placed above the bottom layer of fruit and another set was placed below the top layer. The two sets of sheets in a standard carton contained an average of 2.05 grams of available ammonia.

Ammonia Analysis

The ammonia content of mats or sheets to be used in experiments was determined in quintuplicate to obtain a reasonable average for a particular lot. Either sheets or mats that were removed from cartons of citrus during the course of an experiment were stored with sodium bisulfate in polyethylene bags until analyzed. At that time the contents of the bags were transferred completely to a liter Erlenmeyer flask, and the polyethylene bag was rinsed with distilled water which was added to the flask. Mats to be analyzed were torn apart to release the pellets; both paper and pellets were transferred to a 1-liter Erlenmeyer flask, then 50 ml of 6N sulfuric acid were added to cause decomposition. Sheets of paper containing the ammonium sulfate were cut into narrow strips into a large funnel whose stem extended into the flask. This was done to avoid loss of flakes of ammonium sulfate that fell during cutting. The strips were forced into the flask with a stirring rod and the funnel rinsed with 50 ml of 6N sulfuric acid and water. The flask was then filled to the measured liter mark with distilled water. After the contents of the flask were thoroughly stirred, a 25-ml aliquot was transferred to an 800-ml Kjeldahl flask.

The volume in each Kjeldahl flask was made to about 400 ml, then 15 ml of 50 percent sodium hydroxide solution, a few zinc granules as boiling chips, and anti-foam agent (Dow Corning Antifoam A) were added. The solutions were distilled in the usual Kjeldahl manner; the distillate was collected in a 500-ml Erlenmeyer flask containing 50 ml of 2 percent boric acid solution. The ammonia content was determined by titration with standard sulfuric acid solution to a bromocresol green-methyl red endpoint.

EXPERIMENTS AND RESULTS

Ammonia Injection Into Cartons

Fruit temperatures, as recorded by Ryan thermographs, were 45°, 38°, and 37° F, respectively, on the first, fifth, and ninth days in the iced car. The temperature dropped from 60° to 38° during the first 36 hours and remained near 38° during the simulated transit period. After 10 days the fruits were removed from the iced car and their temperature gradually rose to 62°F over the 9-day storage period.

Inoculation wounds examined on the tenth and nineteenth days showed no enlargement due to the ammonia injection at 1/8, 1/4, or 1/2 gm per carton. Treatments of two or three injections at 3/4 gm per injection caused a very slight darkening and enlargement of the wounds. There was no pitting, discoloration, or any other visibly injurious effects to the fruit surface due to the gassing. However, an occasional fruit located directly in front of the point of ammonia injection showed excessive enlargement and darkening in the artificial wound.

On the tenth day, immediately prior to removing cartons from the iced cars to room storage, samples of spores were taken from the surface of the fruit and tested for their viability. This was done by sampling nine fruits removed from the center carton in each stack of ammonia-treated and non-treated fruit. A moistened, sterile cotton plug was swabbed over the fruit, carefully avoiding the wound and taking care to swab fruit and plates in the same manner. Plates were incubated for 24 hours at 68° F and the growth of spores from each fruit was classified for extent as follows: 0 -- no apparent growth, 1 -- very slight growth which was just visible, 2 -- light growth, 3 -- moderate growth, and 4 -- growth equal to the check. Spore germination counts were also made from 10 fields per plate or 90 fields per treatment. The numerical rat-

ings reflecting growth of many thousands of spores are used here. The averages of ratings called 0, 0.1 to 1.0, 1.1 to 2, 2.1 to 3.0, were equivalent to spore germinations of 2, 9, 26, and 48 percent, respectively; the checks showed 60 percent germination.

On the nineteenth day all treatments were evaluated for decay and only those wounds artificially produced and inoculated were observed and counted. The decay results and spore viability are graphed in Figure 3 for each of the different injection rates.

Figure 3 shows that the 1/8 and 1/4 gm injections are distinctly less effective than the 1/2 and 3/4 gm injections in controlling decay and reducing spore viability. An adequate quantity of gas is, therefore, one important factor in assuring control of decay. Results shown in Figure 3 also suggest that a single injection on the fifth or ninth day at the temperatures of this experiment is more efficient than a single injection on the first day, and that two or three injections are more effective than one. Reduction of decay is also shown to be proportionate to the reduction in spore viability at the four injection quantities used. Two or three injections at 1/2 gm per injection gave good control of decay and a reduction in spore viability; moreover, they produced no injury on the fruit surface and no enlargement of the artificial wounds.

The Relation of Ammonia Emission Patterns to Decay Control

The ammonia tablet mats (Fig. 2) were first tested commercially in June, 1956. Analysis of the tablets for ammonia release under simulated shipping conditions indicated a slow release of the gas during the first few days. To effect an early release of ammonia, one or two of the mats were wetted by dipping in water prior to placement in the carton. The following experiment was conducted to study the release of ammonia from moistened mats compared with unmoistened mats under simulated refrigerator car conditions.

Twenty-five wounded and inoculated Valencia oranges were randomized with non-wounded, non-inoculated fruit into each of 69 vented cartons. Of these, 21 cartons contained two dry tablet mats, both located above the bottom layer of fruit; 21 cartons contained one dry mat and one wet mat, and 21 cartons contained two wet mats, similarly located. The mats were moistened by plunging in water for approximately 4 seconds. Three cartons were prepared with diphenyl-impregnated paper and three others were untreated. This entire procedure was duplicated with lemons in unvented cartons. All 126 cartons of ammonia-treated fruit were placed in one compartment of the iced railroad car in stacks three cartons high, while the 6 diphenyl-treated and 6 untreated cartons of fruit were placed at opposite ends of a second compartment.

On the first day after inoculation the two mats of tablets from each of 3 vented and 3 non-vented cartons of each treatment (2 dry mats, 1 wet mat; 1 dry mat, 2 wet mats) were removed and analyzed for ammonia. This procedure was repeated on the 2nd, 3rd, 4th, 7th, 9th, and 14th days after inoculation, and decayed fruits were counted on the 14th day. Thus, the control of decay by exposure to ammonia at different times and for several periods of exposure was studied. On the ninth day after inoculation the fruit was removed from the iced car to storage at room temperature and the final mats removed from the last group of cartons on the fourteenth day; at this time all of the wounded and inoculated fruits were examined for decay.

Fruit temperatures, determined with a Ryan thermograph inside the carton, dropped from 80° to 50° F the first day, and to 42° the second day, and were at 40° to 42° the remaining 9 days while under ice refrigeration. After 5 days at room storage the fruit temperature rose to 83°.

The average of the quantities of ammonia evolved and the percentage of decay control for each of the treatments are shown in Figure 4. The results shown in Figure 4 indicate that control of decay by the ammonia tablets appears to be a function of the quantity of ammonia released. Note that during the first week very little ammonia was emitted by the dry mats. Wetting of the tablets produced an initial release during the first day, resulting in less ammonia available for release after the ninth day when cartons of fruit were removed from the iced car. As shown by the percentage of decay after 14 days, wetting mats did not increase the effectiveness of the treatment.

Evolution of Ammonia at Controlled Temperatures

From a study of the results of ammonia injection experiments (Fig. 3); of ammonia emission patterns of Winn-Mat tablets, as in Figure 4; and of other formulations tested under simulated iced-car conditions, it became apparent that a formulation might fail to control decay if too little ammonia was released during the first few days, if the fruit temperature was high. Under commercial conditions this might occur if there was delay in icing the railroad cars; spores could germinate and penetrate into a wound before a concentration of ammonia lethal to the fungi

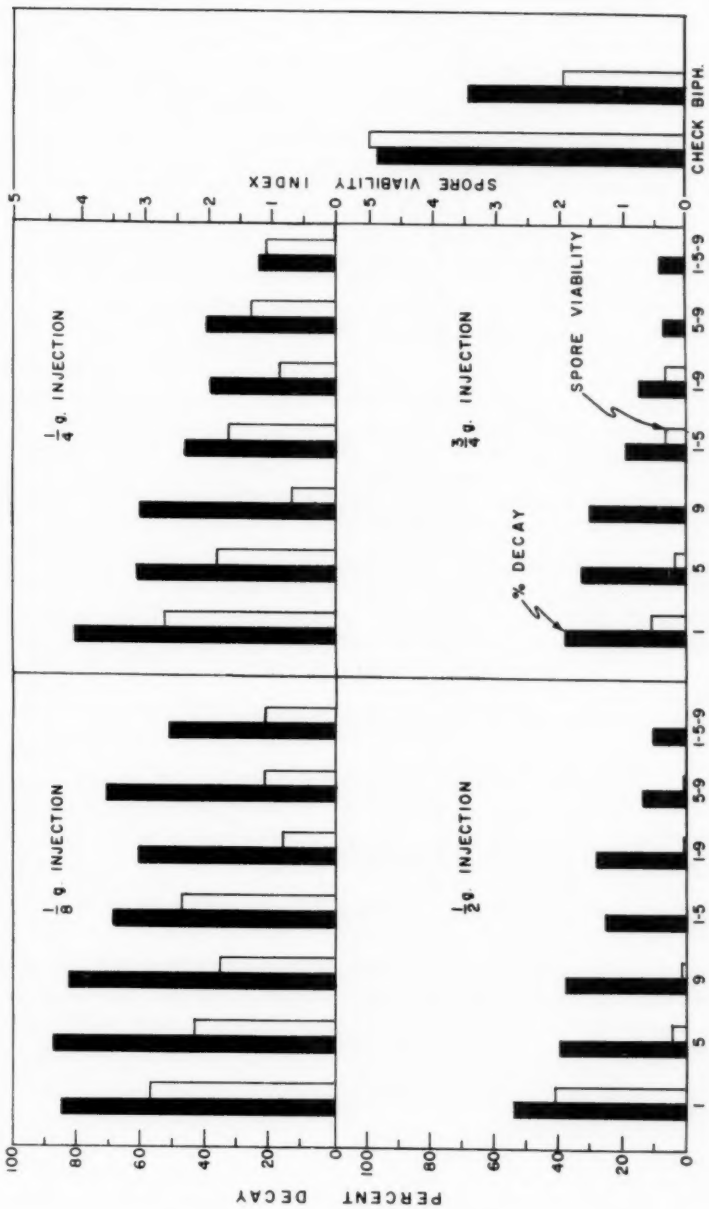

FIGURE 3. Bar graph showing the percentage of decay 19 days after inoculation, and the index of viability of spores on the fruit surface 10 days after inoculation. The cartons of fruit were injected with varying quantities of ammonia gas while under simulated, iced railroad car conditions. Note the reduction of spore viability for the 1/2 and 3/4 gm injections compared with the 1/8 and 1/4 gm injections.

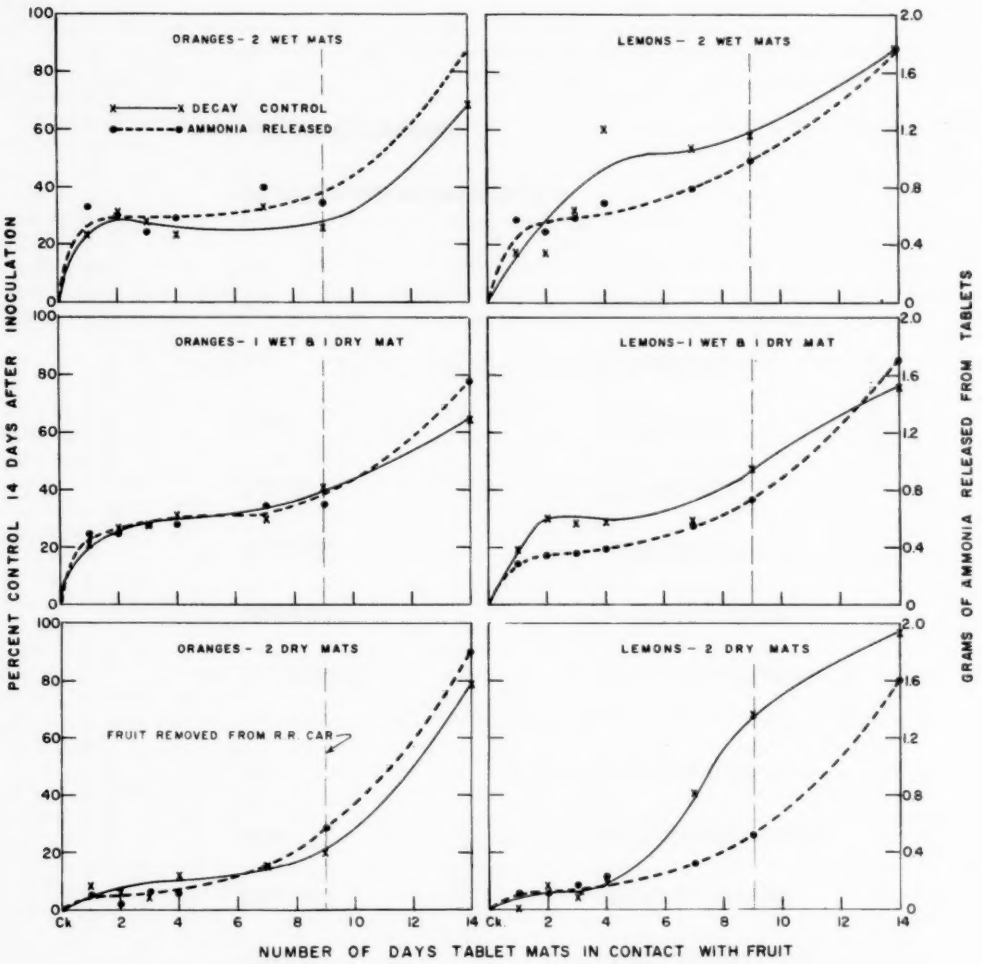

FIGURE 4. The relationship of amount of ammonia released from tablets to the control of decay as recorded 14 days after inoculation. The corresponding points shown for any given day represent the ammonia released (dotted line) up to that day and the control of decay (solid line) resulting 14 days later for that quantity of ammonia released. Note the correlation between the gas released and control of decay. Note also that the wetting of the tablets did not increase the efficiency of treatment.

was released (10). Experiments were therefore planned to determine the ammonia emission patterns of tablets and sheets at three temperature levels: 68°, 50°, and 32°F, and to compare these emission patterns with the resulting control of decay of wounded, inoculated fruit. Cartons about one-third the size of standard commercial cartons were used in these experiments. They measured 6 1/4 inches wide, 9 1/4 inches deep, and 12 inches long and held 40 fruits each. The smaller carton was used in preference to the larger carton to obtain sufficient replication in the limited space of the constant temperature rooms; these cartons were non-vented and provided a uniform internal environment for all treatments.

Valencia oranges were wounded, inoculated, and randomized into the cartons. There were two ammonia treatments, utilizing either tablets or sheets. For the tablets, two separate sections of mats, each holding three tablets (Fig. 2), were placed in the center of the carton. Sheets were placed above the bottom layer and below the top layer of fruit. After the inoculation and treatment with the ammonia-emitting materials, the fruits were stored either at 32° or 50° F. The fruits remained in contact with the ammonia from the generators for various periods of time, that is, for 1, 3, 5, 7, or 9 days. All combinations of these temperatures, time intervals, and the two types of treatment were studied; each combination was replicated 5 times. At the end of each time interval the two mats or sheets from each of the 5 replicates were removed for analysis of residual ammonia. A diphenyl treatment and a check were included at each temperature.

After 7 days the decayed oranges were counted for all lots held at 68° F. After 9 days the fruit held at 50° and 32° were removed to 68° and held at this temperature until decay in the untreated checks had advanced to a point equal to decay in those previously held at 68°. Decay counts were made on the thirteenth and fifteenth days for fruits held at 50° and 32°, respectively.

The tablet mats or sheets, which were removed from the cartons periodically, were analyzed for the ammonia remaining, and the results multiplied by a factor of 3 to enable comparison with standard cartons. Analysis of 6 original tablet mats showed an ammonia content of 2.08, 2.09, 2.05, 2.09, 2.00, and 2.08 gm, for an average of 2.07 gm. The sheets averaged 2.03, 2.03, 1.98, 2.10, 2.04, and 2.12 gm, for an average of 2.05 gm ammonia. The average values for ammonia release determined for sheets and tablets held for the varying periods of time are plotted in Figure 5. When joined by a dotted line, these show the pattern of ammonia release by tablets and sheets for the respective temperatures.

Decay in the untreated checks held at 68°, 50°, and 32° F was 93, 99, and 90 percent, respectively. The percentage control obtained from the ammonia treatments at the three temperatures is shown in Figure 5 by the unbroken line.

The results shown in Figure 5 again demonstrate that the degree of control of decay appears to be a function of the amount of ammonia released (see also Fig. 4). Note the tendency for the ammonia emission and decay control curves to nearly coincide. The sheets released ammonia sooner than the tablets. The tablets emitted ammonia very poorly at all three temperatures during the first 3 days; at 68°F the tablets began emitting gas after the third day when decay was already visible, thus the ammonia was released too late to be effective.

The results of this experiment, shown in Figure 5, were similar to those of an earlier experiment. In both experiments the release of ammonia at 50° F was greater than at 68°, with the control of decay proportional to the amount of ammonia released. Also, in both experiments the release of ammonia at 32° was considerably curtailed compared with that at 50° and 68°; and in both experiments the sheets provided decay control superior to that from the tablets at all the temperature levels investigated.

The tablets used in these experiments released very little ammonia either under iced-car or constant temperature conditions. One method of increasing ammonia released by tablets is by incorporation of deliquescent materials. Of the many such compounds tried, ammonium succinate and calcium chloride were most effective. The addition of 10 percent ammonium succinate to a standard tablet formulation showed an emission pattern with a peak of ammonia release between the fourth and eleventh days under transit conditions, or 36 percent of total available ammonia released compared with 11 percent release for the standard tablets during the same period. Addition of ammonium succinate or calcium chloride has a tendency to produce a wet mat, an effect very undesirable when cartons of fruit are opened for display.

DISCUSSION

The effectiveness of ammonia as a fungicide against the blue-green mold spores on citrus fruit depends upon such interrelated factors as the amount of moisture uptake in the spores prior to fumigation, the sorption of the gas by surrounding materials, the time and quantity of gas in-

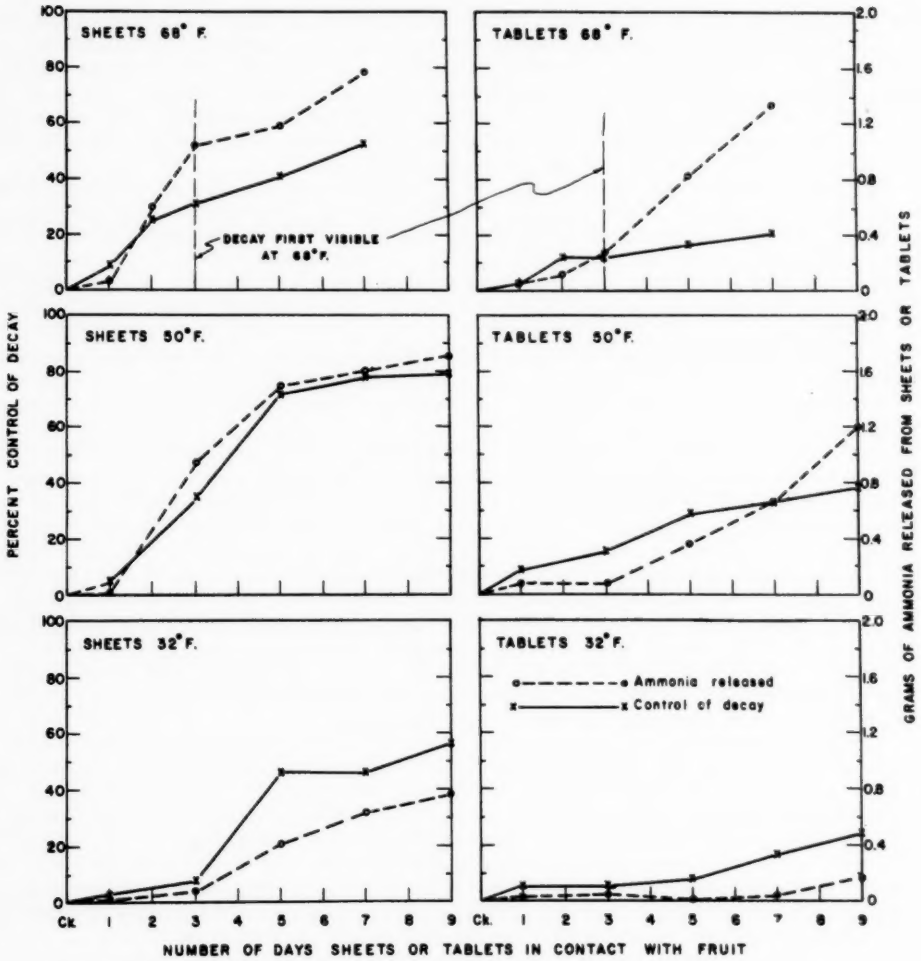
SHEETS 68° F.
TABLETS 68° F.
DECAY FIRST VISIBLE AT 68°F.
SHEETS 50°F.
TABLETS 50°F.
SHEETS 32°F.
TABLETS 32°F.
Ammonia released
Control of decay
PERCENT CONTROL OF DECAY
GRAMS OF AMMONIA RELEASED FROM SHEETS OR TABLETS
Ck. 1 2 3 4 5 6 7 8 9
NUMBER OF DAYS SHEETS OR TABLETS IN CONTACT WITH FRUIT

FIGURE 5. Graphs showing the relationship of ammonia released from tablets and sheets at three temperature levels to the corresponding control of decay on Valencia oranges. Each point for a given day represents ammonia released up to that day and the control of decay resulting at the end of the experiment. Note the inhibition of ammonia release at 32° F, the differences in release by sheets and by tablets, and the relatively good correlation between ammonia released and control of decay.

jected, and the temperature of the fruit. Studies presented in this paper attempt to relate some of these factors to the control of citrus decay.

Preliminary unpublished data[3] indicate that dry spores are less susceptible to destruction by ammonia fumigation than spores which are first preconditioned to a moist environment. The microclimate surrounding spores in a wound has not been studied; however, it can be assumed that the environment favorable to spore swelling and germination would also be favorable for fungicidal effectiveness of ammonia gas.

It was shown (6, 15) that cartons sorbed considerably more ammonia than the fruit. Too small an ammonia concentration might, therefore, fail to control decay due to sorption by the carton, leaving an insufficient ammonia concentration surrounding the fruit. Fungicidal concentrations of the gas (15, 16) must be present over a time period sufficient to contact the spores when they are hydrated or are beginning to germinate and before they have penetrated the fruit rind and are beyond reach of the gas. Failure to control decay by injections of 1/8 and 1/4 gm ammonia may be due to sorption of the gas by the cartons, leaving insufficient concentrations surrounding the fruit.

The timing of injection or of evolution from chemicals placed in a carton of fruit determines the effectiveness of ammonia. This in turn is related to the moisture conditions within the carton and to temperature of the fruit. For example, the delayed release of ammonia by tablets at 68° F, shown in Figure 5, failed to control decay because the fungus had already penetrated the rind of the fruit. Note, however, that in Figure 3 a later injection of gas under cooler conditions was effective in reducing decay.

One factor which has not been sufficiently explored is in-carton moisture as it is related to the efficacy of ammonia injection or evolution from prepared tablets or sheets. Release of ammonia gas from a mixture of an alkali and ammonium sulfate, as formulated in the tablets and sheets and used as ammonia generators, depends upon the humidity and moisture conditions within the cartons. Temperature of the fruit in relation to air temperature and transpiration by the fruit determine the moisture conditions in a carton. The interrelationship of humidity and temperatures within fiberboard cartons was discussed by Harding[4], who demonstrated that the relative humidity inside a carton will decrease when warm fruit is transferred to the cooler atmosphere of an iced car and that relative humidity in cartons increased rapidly when outside temperatures were considerably higher than temperatures within the carton. The gain or loss of water vapor from the interior of the carton is a function of the difference in vapor pressure between the atmosphere inside the carton and that outside the carton, the movement of water vapor going in the direction of lower vapor pressure. Note in Figure 4 that wetted mats released ammonia only during the first day, with a very little additional release from the second through the ninth days. Wetted mats examined after 24 hours under iced refrigeration were observed to be dry. Apparently the moisture from the warmer environment within the carton moved out of the carton into the cooler surrounding atmosphere. Harding demonstrated that after the initial drop of relative humidity within a carton of warm fruit transferred to an iced car, the humidity began to rise, presumably because of equalization of the water vapor pressures inside and outside of the carton as the temperatures equalized, and of the production of moisture by the transpiring fruit. It is this moisture which activates the release of ammonia from tablets or sheets.

Whether ammonia can be used successfully to control decay will depend upon the development of a formulation or combination of formulations and/or gas treatments which will provide an effective but noninjurious quantity of gas at a time when spores of the fungus are most susceptible to the gas.

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SEPTORIA SPOT OF PLUM FRUITS

Paul L. Lentz

Early in June of this year, Dr. P. A. Young, Texas Agricultural Experiment Station, Jacksonville, Texas, sent to the National Fungus Collections several diseased Bruce plum fruits. Each fruit was marred by a number of blue spots that contrasted markedly with the reddish surface of the ripening fruit. The spots (Fig. 1, center) were circular or slightly irregular, 3 to 7 mm in diameter, pale Varley's gray (4), with a vinaceous gray zone of discoloration extending approximately 1 to 2 mm beyond the margins of the spots. Many of the lesions had separated on at least one side from the surrounding epidermis of the fruit. No fruiting structures of fungi were visible on the spots until they were examined under a dissecting microscope. Then as many as 40 exceedingly minute, pale, resinous-appearing masses of conidia were seen to be exuding from inconspicuous fructifications embedded within the superficial tissue of the fruit. The conidia were representative of Septoria. Those assumed to be mature were hyaline, generally 3- to 6-septate and approximately 25-52 x 2-2.5 μ (rarely to 3 μ). Many apparently younger conidia of smaller dimensions and with fewer septa were also observed. A typical conidium was generally curved or flexuous and tapered very gradually from base to apex, where it was very slender but rounded instead of acute.

In the checklist of Weiss and O'Brien (5) S. pruni Ell. and S. purpureocincta Ell. & Ev. are the only species of Septoria reported as pathogenic on plums (Prunus americana Marsh.) in the United States, and these have been reported only as the cause of leaf spots. According to the type description (2), S. purpureocincta causes irregular amphigenous spots characterized by broad dark purple margins on the upper surface of the leaf. The fructifications are formed on the upper leaf surface and bear hyaline, filiform, reportedly non-septate conidia 20-30 x 2 μ. The type specimen was collected on leaves of Prunus americana at Rockport, Kansas, Sept. 1892, by Bartholomew (no. 709). Examination of a specimen bearing these data in the National Fungus Collections reveals that the conidia are definitely septate, some having as many as six septa (Fig. 3). The few other specimens filed as S. purpureocincta (including Ellis and Everhart, North Am. Fungi 3374) also have septate conidia and agree in all other respects with the specimen regarded as part of the type.

The noteworthy blue spots and septate conidia of the plum fruit fungus were very suggestive of S. purpureocincta. It seemed desirable to validate this observation by finding the characteristic spots on the leaves, so Dr. Young obtained some heavily spotted leaves from the orchards in which the diseased fruits had appeared. Though similar in color and in the appearance of the minute spore masses, the spots on these leaves were generally smaller and more regularly circular than the spots produced by S. purpureocinta and had a decided tendency to fall out of the leaves, causing a shot-hole effect. Production of shot-holes in leaves of Prunus spp. has previously been attributed to Cylindrosporium padi Karst. and Septoria cerasina Pk., but both of these differ from the plum fungus in having 1-septate conidia exuded in relatively large amber-colored masses on the lower surface of the leaf. The type specimens were not examined, but it seems likely that C. padi of North American authors and S. cerasina represent a single species. Higgins (3) has previously remarked on the Cylindrosporium-like development of S. cerasina.

According to the original description of S. pruni Ell. (1), the other species reported on plum, the fructifications are formed in deciduous spots 1 to 3 mm in diameter. The cylindrical, curved, obtuse spores are 4- to 6-septate and 30-50 x 2 μ. The specimen of Ellis, North Am. Fungi 1151, in the National Fungus Collections evidently represents a part of the type collection from Lexington, Kentucky, and it agrees in all essential respects with the original description. The plum fungus from Texas seems to be conspecific with this and other specimens of S. pruni in the National Fungus Collections. Except for the slightly larger and more irregular spots with less tendency to fall out of the leaves, specimens of S. purpureocincta also appear to be indistinguishable from those of S. pruni and the two names are regarded as synonymous. Specimens having characteristics of either S. pruni or S. purpureocincta presumably should be named S. pruni Ell. This species has been reported as the cause of plum leaf spots in Iowa, Kansas, Kentucky, Nebraska, and North Dakota (5), but has apparently never before been indicated as the cause of plum fruit spots.

The fructifications of S. pruni, as they develop on the leaves, appear to be somewhat intermediate between acervuli and pycnidia. They seem to originate within a cell of the leaf

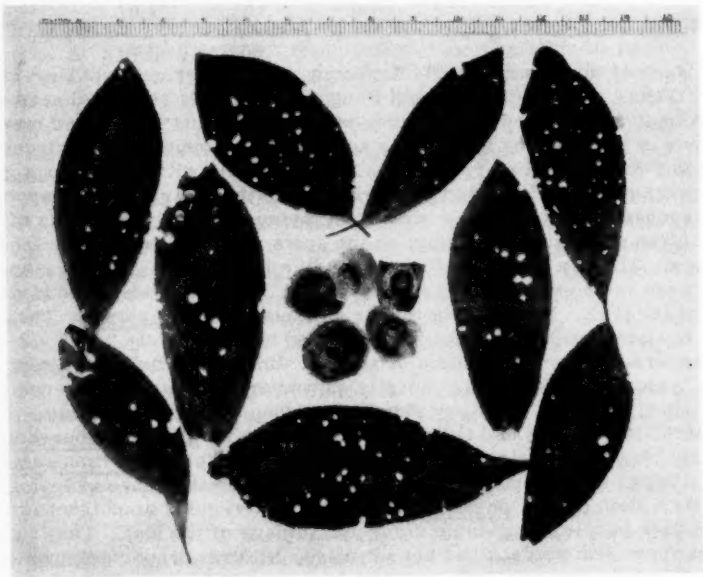

FIGURE 1. Shot-hole caused by Septoria pruni on plum leaves, and spots on several pieces of plum fruit.

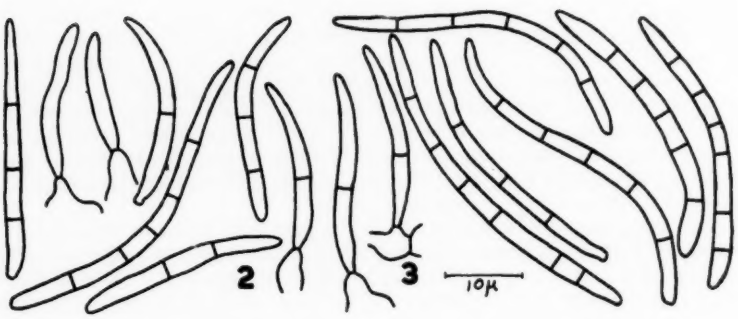

FIGURE 2. Septoria pruni conidia from type collection (Ellis, North Am. Fungi. 1151).

FIGURE 3. Septoria purpureocincta conidia from type collection (Bartholomew 709).

epidermis, then to dislocate the surrounding cells and push aside the apices of the underlying cells of the palisade layer. Eventually the depth of the fructifications may approximate their width, which is about 60 μ. The apical opening is broad in relation to the width of the fructification, but the conidial mass, as seen on the leaf surface, is so small that it can be observed only when magnified. In the fruit spots, the fructifications disrupt several superficial layers of cells as they develop.

According to Dr. Young, the disease has killed many leaves of trees affected in orchards near Jacksonville. The lesions on the plum fruits are very unsightly, and presumably would affect their marketability. In addition, it is likely that the diseased fruits undergo further deterioration as they ripen.

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LOSSES CAUSED BY CROWN RUST OF OATS IN 1956 and 1957[1]

R. M. Endo and G. H. Boewe[2]

The most severe crown rust epiphytotic on oats in 36 years occurred in Illinois in 1957, causing an estimated loss of 27,263,000 bushels in yield or $16,900,000. Winter as well as spring oats were severely damaged. Factors associated with the 1957 epiphytotic and the method for estimating loss are described. In 1956, measurable crown rust losses occurred only in northern Illinois. The bushel loss in yield was estimated at 3,021,000 bushels, or a loss of $2,205,500.

The 1957 Illinois oat crop was severely damaged by crown rust, caused by Puccinia coronata. The 1957 crown rust epiphytotic was the most severe ever recorded by the Illinois Natural History Survey during the 36 years that records have been taken. Both spring and winter oats -- the latter is grown only in southern Illinois and usually escapes severe crown rust infection because of its early maturity -- were heavily damaged. This paper reports some factors associated with the 1957 crown rust epiphytotic, and contrasts the losses that were caused by crown rust during 1956, a relatively mild crown rust year, with the losses in 1957, a very severe crown rust year.

The 1957 epiphytotic was initiated on winter oats in southern Illinois and spread rapidly northward to the spring oat crop. Crown rust was first observed in southern Illinois on May 13, in trace amounts except in centers of infection where the intensity varied from 5 to 10 percent. Secondary spread was evident, indicating that primary infection had occurred during the last 2 weeks of April. On May 21 a field of winter oats in the early heading stage had 9 percent of its leaf area infected, and on June 6 a field of winter oats in the early dough stage had 72 percent of its leaf area infected. These are the most severe rust intensities ever recorded in southern Illinois on winter oats this early in the season.

Circumstantial evidence indicates that windborne urediospores from the States south and southwest of Illinois were responsible for the establishment of primary infection on winter oats in southern Illinois[3]. Crown rust was reported abundant in April on winter oats in Texas, Louisiana, Mississippi, Oklahoma, and Arkansas[4]. Beginning April 15, a very unusual 2-week period of southwesterly and southerly winds from the Gulf region brought abundant rainfall, above-average temperatures (+12° F), and crown rust urediospores to the southern part of the State. Considerable cyclonic and tornado activity also occurred in the Gulf region during this period, a factor which may have helped to carry urediospores aloft into the air stream. The heavy rainfall in Illinois during this period favored not only "fallout" but also germination of the spores. Winter oats responded to the change in the weather with rapid and vigorous growth. Thus, conditions were ideal for the initial establishment of rust on winter oats in southern Illinois.

Heavy rains during April greatly delayed the planting of spring oats in south central and central Illinois. All areas had above-average precipitation during April. In the central and south central areas precipitation was more than double the long term mean. As a result spring oats were planted on normal planting dates in northern Illinois only. According to the Illinois

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[1] Cooperative investigation by the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, the Illinois State Natural History Survey, and the Illinois Agricultural Experiment Station.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Assistant Professor of Plant Pathology, University of Illinois, Urbana, Illinois; Associate Plant Pathologist, Illinois State Natural History Survey, Urbana, Illinois, respectively.

[3] Crown rust urediospores did not overwinter in southern Illinois in 1957; and buckthorn, the alternate host of the crown rust fungus, is very rare and has never been observed to function as a source of primary inoculum in this area.

[4] The crown rust races abundant in these States in 1957 were the same as races identified from Illinois. They comprised mainly races attacking Bond-derivatives (races 202 and 203) and Victoria-derivatives (races 213 and 216). (Data supplied by Dr. M. D. Simons, Ames, Iowa.)

Table 1. Estimated loss due to crown rust of oats in Illinois in 1957.

Section of State	Estimated Loss (Percent)	Acreage	Yield (Bushels)	Potential Yield (Bushels)	Loss (Bushels)
Northwest	9.4	718,100	36,660,400	40,000,100	3,339,700
Northeast	17.1	548,300	25,838,600	31,168,400	5,330,800
West	24.9	269,000	10,299,700	13,714,600	3,414,900
Central	28.9	455,800	14,767,200	20,769,600	6,002,400
East	25.9	435,900	11,554,700	15,593,400	4,038,700
West Southwest	38.8	127,800	3,387,700	5,535,500	2,147,800
East Southeast	38.3	120,600	2,960,000	4,797,400	1,837,300
Southwest	38.4	54,300	1,279,500	2,076,300	796,700
Southeast	39.6	21,200	541,200	896,000	354,700
State	20.3	2,751,000	107,289,000	134,551,300	27,263,000

Weekly Weather and Crop Bulletin report of April 16, 1957, only about 60 percent of the oat crop had been seeded as compared with 96 percent on the same date in 1956. The 1951-1955 average is 70 percent.

During May and June extremely favorable weather (temperature and precipitation) for crown rust development occurred in all areas of the State with the possible exception of northwestern Illinois. During April, May, and June, all crop districts, except the northwestern district, had above-average precipitation ranging from 127 to 205 percent of normal; in the northwestern district precipitation averaged 89 percent of normal, or 10.4 inches, of which only 2.4 inches fell in June. Reduced rainfall may have limited rust development in northwestern Illinois (Table 1) since June is the critical month for rust development in northern Illinois. Also, only 25 percent of the oat crop in the area was planted to susceptible varieties there, in contrast with 50 percent or more in the other districts.
in northwestern Illinois. The district percentage figures were in substantial agreement with those obtained by comparing actual yields of Clinton and Clintland from a limited number of Illinois farms[7].

To estimate the percentage yield loss for the other varieties, it was necessary to calculate the 1957 yield of each variety as it would have been in the absence of crown rust. This was done by comparing the average regional yields (northern, central, and southern regions) of each variety for the years 1953-1956 with the yield of Clintland for the same period[8]. For example, in northern Illinois, the Clarion variety yielded 98 percent as much as Clintland during this period.

An estimate of the damage caused by crown rust in the State was obtained by using the comparative yields of certain oat varieties and backcross derivatives in 52 spring oat varietal demonstration trials conducted by the Department of Agronomy in seven of the nine crop districts[5]. Since the rust intensity varied within Illinois, it was necessary to determine the crown rust damage occurring in each of the crop districts. Since no yield data are available for the west-southwest and southeast districts, those of east-southeast and southwest districts, respectively, were substituted. The difference in yield between Clintland (C.I. 6701) and Clinton (C.I. 4606) was the basis for estimating rust damage for the Clinton variety, since the two varieties apparently differ only in their reaction to crown rust. Clinton was highly susceptible, whereas Clintland was highly resistant, to the predominant races of crown rust (202, 203, 213, and 216) present in Illinois in 1957. Both varieties are highly susceptible to the new races 276 and 290 which appeared late in the season[6]; however, damage caused by these two races was negligible. District losses for the Clinton variety ranged from 50 percent in southern Illinois to 28.7 percent

[5] 1957 Summary of Illinois Spring Variety Demonstrations, by J. W. Pendleton, W. O. Scott, and E. C. Spurrier.

[6] Race data supplied by Dr. M. D. Simons, Ames, Iowa.

[7] Figures were provided by the Illinois Cooperative Crop Reporting Service.

[8] Based on yield figures published in "Spring Oats in Illinois" by C. M. Brown, et al., Circular 754, 1956. The comparison assumes that the yields were valid measurements of each variety's inherent yielding ability and that crown rust and other diseases were not present.

The probable rust-free yield of Clarion in northwestern Illinois in 1957 was calculated by multiplying the average 1957 yield of Clintland in the Agronomy Demonstration Trials in northwestern Illinois by 98 percent. The loss in yield of Clarion for the northwestern district was obtained by subtracting the average yield obtained for the variety in northwestern Illinois in the 1957 Agronomy Demonstration Trials from the calculated rust-free yield of Clarion. The percentage yield loss for the Clarion variety was then calculated, and multiplied by the percentage of the oat acreage that was seeded to Clarion in northwestern Illinois[9] to obtain the percentage loss for this variety for the district. In this way, a percentage yield loss was calculated for all varieties grown in the northwest district. The total yield loss for the district was then calculated by adding the percentage loss figures for all varieties grown in the district. Finally, the expected yield if crown rust had not been present, and the loss in yield due to crown rust were calculated as follows:

$$\text{Expected oat yield in northwestern Illinois on a crop free from rust} = \frac{\text{Actual 1957 oat yield in northwestern Illinois}}{\text{100\% minus total percent crown rust loss in northwestern Illinois}}$$

$$\text{Loss in yield in northwestern Illinois} = \text{Expected oat yield minus actual 1957 yield}$$

The same calculations were made for each of the crop districts and the total loss for Illinois calculated by adding the loss in yield figures for the nine districts.

The percentage loss on all varieties was 20.3 and the loss in bushels was 27,263,000 (Table 1). These losses represent a monetary value of $16,900,000, based on the 1957 average price of 62 cents per bushel. Crown rust losses as calculated by two additional methods were found to be in substantial agreement. The figures are probably conservative, as data obtained from farm advisers indicate that in the central district an average of 13 percent of the oat acreage was harvested or was cut early for hay because of crown rust.

In addition to the loss in yield, the test weight of oats was also greatly reduced. In the varietal demonstration trials, an average difference in test weight of 3.8 pounds occurred between Clintland and Clinton in northern Illinois, 6.9 pounds in central Illinois, and 4.9 pounds in south-central Illinois. The test weights of Clintland and Clinton are substantially the same when crown rust is not a factor. There is a difference of 3 pounds between number 1 and number 2 oats. Thus, market prices as well as feeding values were reduced. Because the straw was weakened, severely rusted oats lodged badly, and additional labor was used at harvest.

A notable feature of the 1957 crown rust epiphytotic was the outstanding performance of the highly resistant variety Clintland. The protection afforded by rust-tolerant and moderately susceptible varieties such as Newton and Nemaha, respectively, was also manifest, but such varieties suffered considerable yield reductions.

In contrast to 1957, the 1956 crop season was characterized by the great variation in crown rust severity. According to data obtained in the Agronomy Demonstration Trials[10], measurable crown rust damage occurred only in northern Illinois, where a 13.7 percent difference in yield occurred between the crown rust-susceptible variety Clinton and the crown rust-resistant variety Clintland in 15 varietal demonstration trials. The yield reduction was calculated as previously described for the 1957 estimate. The estimated reduction in yield in northern Illinois of the spring oat varieties, Clinton and Nemaha, which occupied 25 and 37 percent of the acreage, respectively, was 3.7 percent or a loss of 3,021,000 bushels. This is a money loss of $2,205,500 based on the 1956 average price of 67 cents per bushel. In addition, a 1.7 pound difference in test weights of Clinton and Clintland resulted. The relative magnitude of the losses in northern Illinois is of considerable importance and emphasizes the need for estimating and reporting disease losses in non-epiphytotic as well as epiphytotic years.

CROPS RESEARCH DIVISION, UNITED STATES DEPARTMENT OF AGRICULTURE, AND ILLINOIS STATE NATURAL HISTORY SURVEY, DEPARTMENT OF BOTANY AND PLANT PATHOLOGY

[9] Percent distribution of oat varieties grown on Illinois farms is based on the May 27 report of the Illinois Cooperative Crop Reporting Service.

[10] 1956 Summary of Illinois Spring Oat Variety Demonstrations, by J. W. Pendleton, W. O. Scott, and E. C. Spurrier.

COLOR DISTRIBUTION AS AN INDICATOR OF COVERAGE IN COMMERCIALLY TREATED WHEAT SEED[1]

Laurence H. Purdy[2]

Summary

Seventy-eight lots of commercially treated wheat seed were examined for thoroughness of coverage. Color distribution, as well as a laboratory method of coverage evaluation, was utilized. Results based on color distribution in 18 lots indicated that 25.9 percent of the seed was untreated. Thirty percent of the seed lots that were examined by the laboratory test failed to produce a dark halo around the seed, indicating the absence of germinated bunt spores.

INTRODUCTION

A striking difference in effectiveness in favor of hexachlorobenzene (HCB) over mercury fungicides in controlling soil-borne bunt (Tilletia caries (DC.) Tul.) when applied to wheat seed was demonstrated in experimental plots in 1954 (1). Over a 3-year period, 1955-57, exceedingly good control of soil-borne common bunt with HCB was obtained in regional experimental plots throughout the Pacific Northwest (3, 4, 5). Field trials in 1955 (2) with seed treated commercially with HCB, and a mercury, revealed that HCB provided much better control than the mercury treatments. However, the control obtained with HCB in these commercial trials was not as good as had been obtained in experiments. Likewise, widescale use of HCB on the seed planted for the 1957 crop seemed to indicate that it failed to provide the high degree of smut control obtained experimentally. Thus, commercial use of HCB for the control of seed-borne and soil-borne common bunt of winter wheat has been less successful than in experimental trials. Possible explanations for the apparent failure of HCB in commercial application were investigated, and the results are presented here.

MATERIALS AND METHODS

Representative lots of winter wheat seed were obtained from seed-treating plants in Washington and Oregon in the fall of 1957. These samples represented seed treated with both slurry and drum-type treaters. Forty-two samples were obtained in Washington and 36 samples in Oregon[3]. Thirty-nine lots from Washington and 25 lots from Oregon were treated with various formulations of HCB. The remainder of the samples were treated with mercury fungicides. One of the formulations of HCB (80 percent active ingredient) that had been used contained a water-soluble red dye that clearly marked the treated seed. Samples treated with this material were chosen for coverage determinations. The degree of coverage was determined by distribution of the color on the seed. Three categories of color distribution were established: 1) well colored, the seed colored distinctly and uniformly red; 2) poorly colored, color poorly and irregularly distributed on seed; 3) uncolored, color on the seed undetectable with the unaided eye. Twenty-ml samples of each seed lot were separated into these three categories, and percentages in each were determined.

A laboratory test described by Zogg (6) and modified slightly was used to evaluate the thoroughness of coverage in all seed lots collected. Vermiculite, 4 cm deep, was placed in the bottom of a glass-covered staining dish (12 x 10 x 7 cm) that contained a glass tube (2 x 6 cm) in contact with the bottom of the dish. A 2-cm layer of screened air-dried soil (Palouse silt loam) was placed over the vermiculite and smoothed. The glass tube was raised slightly so that it

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Agricultural Experiment Stations of Idaho, Oregon, and Washington. Scientific Paper No. 1755, Washington Agricultural Experiment Stations, Pullman, Washington.

[2] Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Pullman, Washington.

[3] Collected through the cooperation of W. B. Raymer, Extension Plant Pathologist, Oregon State College, Corvallis, Oregon.

protruded above the soil surface. Screened spores of Tilletia caries were dusted uniformly over the soil surface through one layer of cheesecloth. Three or four seeds of each seed lot were placed on the soil surface and pressed into the soil. Seed treated in the laboratory at the rates of 1/2, 1, and 2 ounces per bushel of 80 percent HCB, as well as untreated seed, were included in each dish for comparison. Water was added through the glass tube until the soil at the soil-vermiculite interface began to take up water. The dish was then covered and incubated at 10°C. After 9 days the spores germinated, causing the soil surface to appear white. Where no germination occurred, the soil surface remained dark. Thus, the presence or absence of a dark halo around each seed served as a measure of the effectiveness of the treating operation.

RESULTS

The results obtained from the separation of 18 seed lots into the three categories of color distribution are presented in Table 1. In general, this tabulation indicates that more uniform distribution of color was obtained with the higher rates of application. The rather poor distribution of color in lots 4, 10, and 33 may possibly be attributed to some error in the treating process. Poor distribution of color at the rate of 1/2 ounce indicates that the amount of fungicide was insufficient to cover the seed uniformly when applied by seed-treating machines. The average of the three groups showed that 25.9 percent of the seed received no detectable treatment.

Table 1. Color distribution in lots of commercially treated wheat seed from Oregon and Washington.

Seed lot number	Ounces per bushel	Percentage of seed: Well colored	Poorly colored	Uncolored
6	1/2	30.8	28.2	41.0
12	1/2	36.8	58.7	4.5
14	1/2	0.0	65.9	34.1
19	1/2	0.0	57.1	42.9
20	1/2	56.1	33.0	10.9
32	1/2	0.0	56.2	43.8
36	1/2	5.6	82.3	12.1
38	1/2	8.8	44.8	46.4
41	1/2	20.8	55.7	23.3
42	1/2	20.5	55.2	24.3
5	3/4	24.3	67.2	8.5
31	3/4	11.6	81.6	6.8
10	1	7.5	74.8	17.7
15	1	46.8	51.3	1.9
18	1	84.1	15.9	0.0
33	1	0.0	32.5	67.3
4	1 1/2	0.0	23.4	76.6
17	2	30.8	64.5	4.7
Average		21.4	52.7	25.9

Results of laboratory tests on spore germination revealed that a distinct halo, a zone of inhibited spore germination, formed around all seeds that were adequately treated. The halo effect was consistent around all seeds from the laboratory sample treated with an 80 percent HCB formulation (Fig. 1) at rates of 1 and 2 ounces per bushel. It was less consistent around the seed treated at 1/2 ounce in the laboratory, although it was quite distinct when present. Less than 25 percent of the commercially treated seed lots inhibited spore germination, as measured

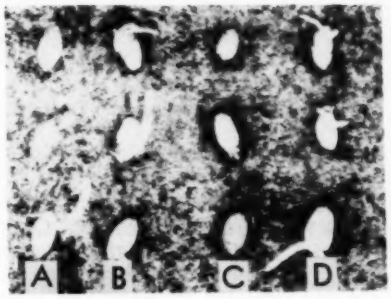

FIGURE 1. Wheat seed on soil surface dusted with bunt spores after 9 days' incubation at 10°C, showing formation of halo around treated seed. Germinated spores on soil surface appear white. A) Untreated seed; B-D seed treated with 80 percent HCB: B) 1/2; C) 1; and D) 2 ounces per bushel.

by the presence of halos around the seed. The remainder reacted according to the degree of coloration. A clearly differentiated halo occurred around well-colored seed but rarely occurred around seed on which color was not detectable. Seed from 30 percent of the seed lots examined failed to produce a halo around the seed.

DISCUSSION

The effectiveness of HCB applied to the seed in controlling soil-borne common bunt in winter wheat depends upon thorough and complete coverage of each seed. The slurry machine, designed on the principle of incomplete coverage with beneficial effects derived from the vapor action of the mercurial fungicide applied, does not apply HCB uniformly on all seeds. Results showed that an average of 25.9 percent of the seeds in treated lots in Oregon and Washington received no treatment on the basis of color distribution as indicative of coverage by the active ingredient. Movement of the fungicide over the seed in the slurry machine is associated with the movement of the water making up the liquid phase of the slurry. Therefore, movement of the water results in movement of the fungicide. Consequently, it would seem valid to assume that coloring of the seed by a dye carried in the water phase is directly indicative of the distribution of the fungicide. This was borne out in the tests on spore germination in the laboratory, in which dark halos, indicating lack of spore germination, appeared around well-colored seed but only very rarely around poorly colored or uncolored seed from the same seed lot.

The laboratory tests reported show that spore germination is inhibited in a limited zone around the seed if treatment of the seed is uniform and coverage is complete. In field plots, more consistent control of soil-borne bunt has been obtained with the 1-ounce rate of 80 percent HCB than with the 1/2-ounce rate. Thus, there is a good correlation between inhibition of spore germination around treated seed in the laboratory and actual control in the field.

On the basis of the results reported here in which 25.9 percent of the seed was either untreated or inadequately treated, the apparent failure of HCB in commercial usage can be attributed to faulty treating. It is apparent that methods of application that will give complete coverage of the seed must be developed if the maximum potential of HCB is to be realized in controlling wheat smut.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, AND THE AGRICULTURAL EXPERIMENT STATIONS OF IDAHO, OREGON, AND WASHINGTON

HEAD SMUT OF SORGHUM AND VARIETAL REACTION

Robert B. Stewart and Lucas Reyes

Abstract

The sorghum head smut problem in the Coastal Bend area of Texas may be attributed primarily to the widespread planting of susceptible varieties and hybrids in this area. Among these, Martin, Texioca 54, Combine 7078, and RS 610 are prominent.

Resistant hybrids and varieties are available through the regular seed sources and the tests of breeding lines indicates a substantial reserve of resistant material. New resistant hybrids seem particularly feasible. RS 630, Caprock, Plainsman, Redbine 66, feterita, and hegari are among the most resistant sorghums available commercially.

Greenhouse evaluation of sorghum for head smut cannot be efficiently or effectively carried out at College Station, Texas except during the winter months. The Panhandle area of Texas would seem to be more nearly suited to this type of testing.

INTRODUCTION

During the past few years, head smut of sorghum (caused by Sorosporium reilianum (Kuehn) McAlpine) has become increasingly severe in the Coastal Bend of Texas. In certain counties of this area, the disease is a limiting factor in sorghum production. A survey of County Agents in 1957 showed that some sorghum plantings were as high as 60 percent infected, and that sorghum fields commonly were 10 to 15 per cent infected[1]. A similar pattern was observed again in 1958.

In regard to the distribution of the disease, it is interesting to note that in 1920 Taubenhaus (9) reported head smut as prevalent in Texas, especially in the Panhandle, and Reed and Melchers (7) stated that it occurred regularly each year at the Amarillo Field Station; whereas today the disease is concentrated in the Coastal Bend and is not a serious problem in the Panhandle.

A search of the literature has not revealed any up-to-date information on varietal reaction to head smut. Taubenhaus (9) reported that milo was immune to head smut, that kafir and broom corn were very susceptible, and that the disease was not serious on sorgo. Reed and Melchers (7) tested 61 individual varieties over a 3-year period and reported milo, feterita, and broom corn to be very resistant. Very little head smut was found among the kafirs and kaoliangs, and with two exceptions the durras were very resistant, whereas the sorgos as a group were only slightly infected. According to Leukel, et al. (4), head smut seems to be severely damaging only to sorgos, durras, and varieties developed from hybrids with these two sorghum groups. Kafirs and sudan grass are only moderately susceptible, while milo, feterita, and broom corn are seldom, if ever, attacked. Reed et al. (8) reported sweet sorghum and sorgo varieties relatively susceptible and feterita, milo, broom corn, kafir, and kaoliang resistant.

As today's hybrids and varieties no longer clearly fit into these broad sorghum groups, these classifications are no longer applicable to individual commercial varieties and hybrids. For this reason and because of conflicts between the reported data and field observations, the reaction of currently used varieties and hybrids have been investigated during the past 2 years.

MATERIALS AND METHODS

Field Tests

Because of the large number of varieties, hybrids, and strains of sorghum to be evaluated, the tests were conducted under field conditions. For these tests, uniformly infested fields were selected in the Coastal Bend area. In 1957 the test was planted near Robstown, Texas. Sorghum grown on this field the previous season was estimated to be 50 percent infected with

[1]Survey conducted by H. E. Smith, Plant Pathologist, Texas Agricultural Extension Service.

head smut. In 1958 a more extensive test was planted near Refugio, Texas, in a field which had shown 30 percent infected plants the previous season. Only natural inoculum was used, and the sorghum was planted at the same time farmers of the locality planted the adjoining fields. The 1958 data from Robstown were taken from a sorghum variety test growing in the same field as the 1957 head smut test (Table 1)[2].

The varieties and hybrids that were extensively tested are commonly grown in the Coastal Bend or have possibilities for this area. A number of breeding strains were tested for only one season. All of the sorghum entries were provided by J. R. Quinby or N. W. Kramer of the Texas Agricultural Experiment Station.

Only actually smutted plants were classed as infected. Blasted head, a common symptom of smutted sorghum, was disregarded because of possible confusion with blasting due to other environmental factors. However, the number of blasted heads was very small in all these tests and their exclusion or inclusion would not significantly affect the data obtained. In the randomized block test, each entry was represented by 400 to 700 plants. The other entries were represented as indicated in Tables 1 and 3.

Greenhouse Tests

Space limitation makes greenhouse testing of large numbers of strains, hybrids, and varieties impractical. However, for critical evaluation of the better entries in a screening program, an inoculation technique that consistently produces infection in more than 50 percent of a susceptible line (near the maximum in our field experiments) was desired. The literature indicates (1, 3, 8) that securing artificial epiphytotics with head smut is a relatively simple procedure. However, under our conditions, this was not always the case. Accordingly, attempts were made to find a successful inoculation method. Although the techniques tested are not comparable in a strictly experimental sense, the conclusions reached are believed to have some value in that the observations have been made from repeated attempts over a 2-year period to develop an acceptable inoculation method for securing artificial epiphytotics.

The following inoculation procedures were used:

1. Seed were dropped in soil-filled greenhouse benches and liberally sprinkled with inoculum before covering. After emergence, the seedlings were watered twice with a teliospore suspension.
2. Seedlings were allowed to develop to the four-leaf stage in a teliospore-soil mixture (1/3 spores to 2/3 soil by volume) then transplanted into pots and allowed to mature.
3. Seedlings were inoculated in the three- to four-leaf stage by injecting an aqueous teliospore suspension into the growing point area with a hypodermic syringe.
4. Hypodermic injections of sporidial suspensions into the growing point area.
5. Aqueous teliospore suspensions were introduced into the leaf whorl at the three- to four-leaf stage.
6. Seed were subjected to a partial vacuum in an aqueous teliospore suspension and then planted immediately.
7. Seed were stirred in an aqueous teliospore suspension in a Waring Blendor, the excess water was drained and the inoculated seed were held overnight in a moisture-proof plastic bag and planted the following day.
8. Seed were mixed with a liberal portion of dry spores and then planted.

In all cases inoculum was prepared by passing smutted sorghum heads through a Wiley mill with a medium mesh screen. Combine 7078 was used as the test variety.

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[2]Collection of these data was made possible through the courtesy of Mr. R. E. Nolan, County Agricultural Agent, Nueces County, Texas.

RESULTS

Field Tests

Sorghum varieties and hybrids that are commonly grown in the Coastal Bend or that have good possibilities for this area were tested for 2 years in a total of three tests for resistance to head smut. The results from these tests are shown in Tables 1 and 2. It is apparent that good quality sorghum with high resistance to head smut is available for the Coastal Bend area.

Among the hybrids, RS 630 had outstanding resistance. No smut was found in this hybrid. Texas 620 was next in resistance, with a grand average of 4.4 percent infection for the three tests. RS 610 was the most susceptible of the hybrids, with 19.6 percent of the plants infected.

The varieties also showed considerable divergence in reaction. Combine white feterita, Plainsman, Hegari, and Redbine-66 were among the most resistant, whereas Combine 7078 and Combine Shallu were the most susceptible.

An analysis of variance was made for the 20 sorghum varieties and hybrids planted in the replicated test at Refugio and the entries grouped into homogeneous groups (Table 2). Continuous lines connect groups which may be considered to have the same reaction.

Table 3 shows the reaction of 90 selected strains to head smut. Here, as in the hybrid and variety tests, the percentage of infection ranged from 0 to approximately 50. The numerous low ratings indicate a substantial reserve of resistant breeding material. Twenty-nine of the entries developed only 3 percent or less infection and 13 showed no infection. Entries in the randomized block test (Table 2) at Refugio behaved very consistently in each replication. The test had a standard error of 0.86. The consistent performance in this replicated test suggests that the reaction noted for the non-replicated entries is fairly reliable since they were grown in adjacent blocks of the same field.

Greenhouse Tests

The techniques described under Materials and Methods for the greenhouse inoculation experiments can be divided into essentially three methods, these being, seed, soil, and seedling inoculation. Of these methods, only the soil mixture methods (Numbers 1 and 2 in Materials and Methods) produced consistently heavy infection. The best method was to allow seedlings to develop to the four-leaf stage in a spore-soil mixture (1/3 spores and 2/3 soil), then transplant to pots to continue their development. Infections of 80 to 100 percent were obtained with Combine 7078 using this technique. This is essentially the method of Reed et al. (8) and is recommended as a very practical and consistently effective method of greenhouse inoculation.

Under conditions at College Station, Texas, it was possible to develop epiphytotics in the greenhouse only during the winter months. This is doubtless because infection by Sorosporium reilianum is drastically curtailed by temperatures above 30° C (1), and it is not practical to maintain greenhouse temperatures below this level in the spring, summer, and fall.

DISCUSSION

As indicated previously, the current head smut problem in Texas can best be met with resistant varieties. It is also apparent that excellent resistance is already present in certain commercially available varieties and hybrids. Furthermore, the problem appears to be simplified by the increasing use of hybrids. This is evidenced by the fact that RS 630, a hybrid derived from the susceptible female parent Combine kafir-60 (9.3 percent infected) and Combine white feterita (0.0 percent infected) is as resistant as its male parent (0.0 percent infected). There appears to be complete dominance of resistance in this cross. It would seem feasible that other desirable hybrids could be developed by utilizing parents with a similar high degree of resistance.

Another type of resistance also seems to be present. This is evidenced by the fact that only one of the hybrids (RS 610) is more susceptible than its common parent Combine kafir-60, whereas RS 650, Texas 620, and Texas 611 were significantly more resistant, and Texas 601, Texas 660, and RS 590 did not differ (Table 2) significantly from Combine kafir-60. Although these data do not permit more than speculation as to the mode of inheritance of this type of resistance, it is probable that it is not a qualitative character, because RS 650, Texas 620, and Texas 611 are all more resistant than Combine kafir-60 (9.3 percent infected) but their male parents Plainsman 7005 (= R7005 Plainsman), Tx 07 (= R5507 Tx 07), and Tx 74 (= R5874 Tx

Table 1. The reaction of selected sorghum varieties and hybrids to head smut.

	Robstown 1957		Robstown 1958		Refugio 1958		
	Number Plants	Per cent Infected	Number Plants	Per cent Infected	Number Plants	Per cent Infected	Average Per cent Infection
Texas 601	104	3.8	225	11.1	721	7.4	7.4
RS 610	229	13.9	274	27.0	420	17.8	19.6
Texas 611	107	10.2	238	15.9	625	5.0	10.4
Texas 620	171	4.7	207	3.4	548	5.1	4.4
RS 630	249	0.0	200	0.0	419	0.0	0.0
RS 650	205	8.8	209	4.3	655	5.0	6.0
Texas 660	77	12.0	217	7.8	572	7.6	9.1
RS 590	106	9.4	217	7.8	586	10.1	9.1
Combine Hegari	---	---	200	0.0	324	0.0	0.0
DD Yellow Sooner Milo	300	1.3	---	---	437	4.5	2.9
Combine 7078	145	22.7	300	36.8	450	37.9	32.5
Combine Shallu	443	31.3	231	13.4	700	58.2	34.3
Redbine-58	114	6.1	212	5.6	---	---	5.9
Redbine-60	59	23.7	218	8.3	445	13.4	15.4
Redbine-66	121	2.5	201	0.5	---	---	1.5
Combine kafir-60	66	12.1	210	4.7	622	9.3	8.7
Martin	149	7.4	215	6.9	515	3.6	5.9
Texioca-54	---	---	210	4.7	528	10.4	7.6
Plainsman	---	---	200	0.0	533	2.3	1.2

Table 2. Twenty sorghum hybrids and varieties grouped as to head smut resistance.*

Entries	Mean Per cent Infected
Combine White feterita	0.0
RS 630	0.0
Plainsman	2.3
Martin	3.6
RS 650	5.0
Texas 620	5.1
Texas 611	5.5
H 7308	6.5
Texas 601	7.4
Texas 660	7.6
DeKalb C44a	7.8
Combine kafir-60	9.3
RS 590	10.1
Texloca 54	10.4
Redbine-60	13.4
AMAK R-10	13.5
DeKalb E56a	15.0
RS 610	17.8
Combine 7078	37.9
Combine shallu	52.2

*Analysis according to Duncan's multiple range and multiple F tests. Significant differences (1% level) are indicated by the breaks in the horizontal lines.

Table 3. The reaction of selected sorghum strains to head smut (Refugio, Texas, 1958).

Strains	Number Plants	Percent Infected	Strains	Number Plants	Percent Infected
R6489 Norghum	185	1.6	B385 Combine kafir	145	5.5
R386 Redbine-60	114	7.8	B3197 Combine kafir	136	7.3
R5904 Tx 04	162	1.2	B600 Combine kafir	97	10.3
R7078 Combine 7078	115	36.0	B601 Combine kafir	119	10.9
R5874 Tx 74	150	22.0	B602 Combine kafir	81	8.6
R5507 Tx 07	126	0.7	B603 Combine kafir	121	11.5
R396 Tx 09	62	0.0	B604 Combine kafir	37	10.8
R7005 Plainsman	115	3.4	B605 Combine kafir	47	17.7
R7000 Caprock	114	0.0	B6062-1-6 Combine kafir	136	0.7
R38311 Day Selection	184	7.0	B6062-1-20 Combine kafir	158	11.3
R40136 Resistant Colby	144	13.8	B6062-14 Combine kafir	189	10.5
R3001-1 Resistant Day	136	22.7	B5765-10-6-3-4 Combine kafir	173	3.4
R38-16 Club Day	57	3.5	B5765-12-4-Combine kafir	178	2.8
R38-2 Club Day	95	1.0	B5765-13-2-1-10-2 Combine kafir	141	7.8
R38-33 Club Day	106	39.6	B5878-4-8-1-5 Combine kafir	164	9.1
R3007-3 Day X Dalhart Res. Wheatland	132	20.4	B5900-18-3-1 Combine kafir	146	12.3
R3056 Redbine Selection	80	8.7	B5900-19-7-1-2 Combine kafir	114	16.6
R5875-6-1-1-1 Texioca 54	185	16.4	B390 Texas Bh. kafir	155	2.5
R3002-1 (Day-DDWS)-4-dw	112	3.5	B6920 Wh. Martin	178	7.8
R3006-1 Breeding Strain	74	1.3	B1740 Edwards Wh. Combine	142	2.8
B398 Martin	152	5.9	FC13490 Straightneck	130	11.5
B399 Resist. Wheatland	107	2.8	FC6604 White African	134	11.9
B378 Redlan	127	5.5	FC9112 Atlas	141	4.4
B387 Redbine-66	149	2.0	CI293 Shantung Br. kaoliang	82	7.3
B3029 Redbine Selection	195	13.3	CI171 Manchu Br. kaoliang	120	0.0
B3042 Redbine Selection	167	10.6	B399 Wheatland	131	2.2
B3044 Redbine Selection	117	1.7	SA7000 Caprock	40	0.0
B3045 Redbine Selection	100	2.0	B605 Combine kafir	154	5.0
B3046 Redbine Selection	137	0.7	Early hegari*	398	0.0
B3047 Redbine Selection	165	0.0	Nebraska 5* =(RS 501)	182	10.4
FC6601 Spur feterita	82	0.0	Hybrid 6511*	68	17.0
SA292 DDY milo	74	0.0	Hybrid 6518*	138	18.8
SA392 Combine hegari	178	0.0	Lindsay H3003**	247	19.0
CI616 Schrock	157	26.4	Lindsay 2919**	No stand	
SA301 Darso No. 28	159	47.0	Redbine SA7185-1-1-3-2**	200	0.0
CI398 White durra	No stand		Redbine SA5507-3-1-4-4-2-9-5-4-5**	200	0.0
FC16207 Kalo	121	3.3	DD Hegari SA7238-2-M-M**	200	0.0
FC8728 Chinese Amber	164	8.5	DD Hegari SA7598**	200	0.0
SA20 Orange	182	9.8	Asgrow AMAK R-12**	256	21.8
FC13498 Saccaline	59	22.9	Sudan grain grass SA6566-1-8-4-3-1-3**	240	16.6
AMAK R-10	416	13.5	Sudan Grain Grass SA7399-3-2-1-1**	241	17.0
Sumac PI35038	413	24.0	Sudan Grain Grass SA6566-1-8-4-3-1-3-10**	213	6.1
Honey SA1664	110	15.0	Sudan Grain Grass SA6657-1-5-12-230 1-2-2-2**		13.0
Hegari PI34911	324	0.0	Hybrid 7853**	225	17.7
Agros 2650 Shallu	294	46.3	Hybrid 7308	320	6.5

*Planted at Robstown 1957
**Planted at Robstown 1958

74) showed 3.4, 0.7, and 22 percent infection, respectively (Table 3). Similarly, Texas 601, Texas 660 and RS 590 had male parents Tx 04 (= R5904 Tx 04), Sa 7000 (= R7000 Caprock), and SA 386 (= R386 Redbine 60), showing 1.2, 0.0, and 7.8 percent (Table 3). RS 610, the only hybrid significantly more susceptible to head smut than its female parent, has a very susceptible variety for its male parent, Combine 7078 (37.9 percent infected). It is noteworthy that all of the hybrids, with the exception of RS 610, are as resistant as or more resistant than their common parent, and that the pollinators, with the exception of Tx 74 and Combine 7078, are more resistant than Combine kafir-60.

The source of the inheritance of this resistance is also obscure. Both milo and kafir are represented in the pedigree of parental types, and both of these types offer resistant lines (Table 3). Some insight into the problem is offered by Marcy's (5) explanation that the same factors, but with reversed effect, govern the reaction of sorghum to Sphacelotheca sorghi (Lk.) Clinton and S. cruenta (Kuehn) Potter. If this relationship were extended to include Sorosporium reilianum, the inheritance of resistance to head smut may be similar to that for loose smut, that is, the feterita-type resistance exhibiting complete dominance and the milo or kafir type exhibiting a two-factor interaction. However, this, too, is mere speculation. Investigation of the inheritance of resistance has been hampered in the past by the difficulty of obtaining large sorghum populations for study. It is evident that sorghum hybrids lend themselves to studies of this nature and that an extension of this study to include further generations could resolve the question of inheritance with relative ease.

Among the more popular grain sorghum varieties Caprock, Plainsman, Redbine 66, Wheatland, and DDY milo showed very good resistance. As all of these varieties have milo in their pedigree it seems likely that the resistance is primarily derived from milo, although individual lines of kafir as well as all the other sorghum groups tested differed greatly in their reaction to head smut.

All previous workers (4, 6, 7, 8, 9) have regarded milo as immune or very highly resistant to head smut. The present test included only two lines that might be regarded as true milos, DDY milo (SA 292) and DDY sooner milo (SA 370). DDY milo showed no head smut but DDY sooner milo showed 4.5 percent infection, indicating that not all milo types are immune.

Feterita also has been agreed upon as immune or highly resistant (4, 6, 7, 8, 9). No smut has been found in feterita in the present test. The results of this test are in agreement with these previous reports.

Kaoliangs are reported as highly resistant by Reed and Melchers (7) and Reed et al. (8). Of the two kaoliang entries in the present test one appeared very resistant and one moderately susceptible.

Sorgo was classed as slightly susceptible by Taubenhaus (9) and Reed and Melchers (7), but Leukel et al. (4) and Reed et al. (8) classed it as very susceptible. The present tests indicate that while sorgos as a group are very susceptible, individual varieties may be resistant.

Similar conflicts are noted for previous records on kafir. Taubenhaus (9) classed kafir as very susceptible, whereas Reed and Melchers (7), Leukel et al. (4), and Reed et al. (8) classed it as slightly susceptible to resistant. The present study more nearly agrees with Taubenhaus, although here, too, individual strains and varieties differ widely.

Durra, according to Leukel et al. (4) is very susceptible, but according to Reed and Melchers (7) it is very resistant with two exceptions. The present test included only one durra type and it failed to produce a satisfactory stand. However, of the few plants which developed, 50 percent were smutted.

The one shallu entry in the tests of Reed and Melchers (7) was found to be only slightly susceptible, whereas shallu in our tests was the most susceptible group.

Sudan grass is reported by Leukel et al. (4) to be only slightly susceptible. Head smut is also reported (10) to occur on Johnson grass. The present test did not include grass sorghums but three lines of Sudan grain-grass were shown to be susceptible.

There do not appear to be any published reports on the reaction of hegari. In the present test, hegari was resistant in all trials. In 1957 the area of the test field near Robstown, Texas that was not planted to the head smut test was seeded to hegari. No head smut was seen in this hegari, although Combine 7078 was 50 percent smutted in the previous season and 22 percent smutted in the current season.

Some of the causes of the recent outbreak of head smut in Texas are apparent from this study. The varieties and hybrids which are the most popular in the Coastal Bend area are also the most susceptible of the common grain sorghums to head smut. Continued growing of these high yielding but susceptible varieties in this area will probably intensify the problem. Although it is not known how long head smut spores can live in the soil, they may live several

years in herbarium material (2) and it is possible they may persist more than one year in the soil. (Experiments are now under way to determine this.) Also, the writer has observed head smut to overwinter in volunteer sorghum in the Robstown, Texas area. The first factor mentioned is of unquestioned importance, but the other factors need further study to determine their importance.

Only two physiological races of head smut are reported (8), one on corn and one on sorghum. It appears likely that more than one race exists in sorghum head smut. The presence of more than one physiological race on sorghum could explain the areas of conflict between this report and previous reports relative to the increased virulence of the organism on some of the standard varieties such as Martin and DDY sooner milo. However, a part of the conflict doubtless arises from the inherent error of generalizing about so heterogeneous a unit as a sorghum group, and from environmental differences. Nonetheless, there persists an indication of more than one physiological race of head smut in sorghum.

The tests at College Station, Texas showed that sorghum head smut evaluation in the greenhouse was a thoroughly practical and efficient method. However, it was possible to carry on such tests only in the winter months due to high temperatures that prevail during the spring, summer, and fall seasons.

Although Leukel (3) has shown that the head smut organism may infect sorghum at any stage of growth, indications are that infection usually takes place during the seedling phase and that temperature and moisture levels are critical factors in the infection incidence (1, 8). This indicates that sorghum planted in a spore-soil mixture in a temperature-controlled growth chamber and allowed to develop to a three- to four-leaf stage might be expected to become smutted even if the plants were later exposed to higher temperatures. If successful, this procedure would permit year-round testing with head smut. When sorghum so treated was planted in 3-gallon clay pots and allowed to grow during July, smut failed to develop. Since it was not possible to grow sorghum to maturity in this particular growth chamber and since no histological observations were made, the source of the difficulty is not apparent. However, it is believed that high temperatures even after infection has taken place may inhibit the development of the parasite.

Regardless of the success of these methods, it is apparent that to extend the testing period in greenhouses in the College Station area will be a more difficult procedure than to move the testing to an area with a longer cool season. Perhaps the best solution to the greenhouse temperature problem would be to do such testing in an area where the longer cool season will permit economical maintaining of greenhouse temperature at 25° to 30° C for longer periods.

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BROWN PATCH OF ST. AUGUSTINE GRASS

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Abstract

Brown patch, a destructive disease of lawns of St. Augustine grass (Stenotaphrum secundatum (Walt.) Kuntze) has been known in Louisiana and other Deep South States for many years, but its etiology had not been definitely established. The present paper presents experimental evidence to show that the disease is caused by Rhizoctonia solani Kuehn (Pellicularia filamentosa (Pat.) Rogers). The following fungicides were tested at different rates for possible control of the disease; Cadminate (cadmium succinate, 60 per cent); Caloclor (mercurous chloride 60 per cent, mercuric chloride 30 per cent); Crag Turf Fungicide 531 (complex compound of cadmium, calcium, copper, zinc sulfates-chromates); Kromad (tetramethylthiuram disulfide 16 per cent, cadmium sebacate 5 per cent, potassium chromate 5 per cent, malachite green 1 per cent, auramine 0.5 percent); Puratized 177 (phenyl amino cadmium dilactate 100 per cent; Puratized Agricultural Spray (phenyl mercuritriethanol ammonium lactate 7.5 per cent; Semesan Turf Fungicide (hydroxymercurichlorophenol 25.3 percent); Tersan (tetramethylthiuram disulfide 75 per cent); and Terraclor (pentachloronitrobenzene 75 per cent WP). Terraclor gave perfect control of the disease in every test (31 separate tests) at all rates ranging from 20 g to 136 g per 100 square feet. At the higher rates (above 100 g per 100 square feet) it was phytotoxic. Puratized 177, at rates of 10 g and 20 g per 100 square feet, also gave perfect control in two trials, but it was not available for further tests. Puratized Agricultural Spray gave good control in one test, but proved ineffective and phytotoxic in three other trials. All the other fungicides were ineffective at the rates tested.

INTRODUCTION

St. Augustine grass (Stenotaphrum secundatum) is one of the most popular lawn grasses in Louisiana. It has many desirable qualities. It forms a dense resilient turf that is better able to stand up under use than most other lawn grasses. It grows well both in direct sunshine and in partial shade, such as under large trees. Also, it remains green throughout the winter, at least in southern Louisiana. With the rapid increase in population and the creation of many new lawns in Louisiana, the demand for St. Augustine grass for lawns is increasing.

St. Augustine grass is not exempt from serious troubles, however. It is subject to several insect pests and fungus diseases. Of the insect pests, chinch bug (Blissus insularis) is by far the most important, being capable of causing severe damage to lawns. Two fungus diseases, leaf spot (caused by two different fungi, Piricularia grisea, and Curvularia sp.) and brown patch (caused by Rhizoctonia solani Kuehn) are very prevalent and often destructive. Of the fungus diseases brown patch, the subject of this paper, is by far the most destructive. It is not a new disease on this host, having been present in Louisiana and other States in the Deep South for many years although its etiology has not been definitely understood. Altstatt (1) in 1942 listed the cause of brown patch as Rhizoctonia solani Kuehn but offered no evidence in support of this statement.

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DESCRIPTION

Brown patch of St. Augustine grass is characterized by the dying of patches of grass in a lawn. These patches, which vary greatly in size from a few square feet to several hundred square feet, are conspicuous from a distance because of the sharp contrast between the dead brown grass in the affected area and the healthy grass turf of the bordering unaffected area of the lawn (Fig. 1). Since the killed turf collapses the affected grass appears sunken in comparison with the surrounding healthy turf. The margin of the advancing brown patch, a belt of moribund grass 2 to 3 inches in width, is smoky yellow in color. Isolated sprigs of healthy grass are almost always found scattered within the area of killed grass. In most cases, the affected areas are circular in outline because the disease, starting as a small spot, advances in all directions at approximately the same rate, thus forming a more or less regular circle. However, if the infection starts close to an obstacle such as a concrete walk or clump of shrubbery, or if patches from two or more centers of infection coalesce, the shape of the affected area may be very irregular.

In southern Louisiana, the disease generally occurs from late September or early October to about the end of May, and is particularly active during the fall and spring months. The rate of spread varies with the time when infection takes place. If infection starts in early spring or fall spread is rapid. A spot 2 or 3 inches in diameter may enlarge to cover an area several feet in diameter in 2 or 3 weeks. On the other hand, when infection starts in late fall or early winter spread is rather slow until the advent of warmer weather in the spring when the rate of spread increases. Late spring infections spread rapidly until the hot weather of late May or early June brings the disease to a standstill. The rare summer infections spread slowly until fall, when the rate of spread increases. The grass reestablishes itself somewhat in the killed areas of the lawn during summer, but when cool weather returns in fall the disease resumes its activity.

In general, the disease most often occurs on flat lawns on which water stands during heavy rains. However, cases have been noted of occurrence on sloping well-drained areas but not on low poorly-drained areas of a particular lawn. Surveys in Baton Rouge and New Orleans show that occurrence is sporadic. Brown patch will sometimes be found only on a single lawn and all other neighboring lawns will be completely free from it, while in other instances practically every lawn in a particular neighborhood will be affected.

Superficially, brown patch resembles the damage done by chinch bugs; both the fungus and chinch bugs kill the grass in patches. Brown patch may start in any part of the lawn and usually starts somewhere away from the borders. Chinch bugs, on the other hand, being migrating insects (crawling, not flying), almost invariably attack the grass at the borders. Chinch bugs, of course, can be seen with the naked eye.

A close look at sprigs of brown-patch affected grass shows that the undersides of the bases of fascicles of sheaths, where they are attached to the stolons, are discolored, soft, and slimy (Fig. 2). Diseased fascicles are easily broken off from the stolon. Microscopic examination of cross sections of newly infected sheaths reveals large collapsed areas within the parenchymatous tissue. The vascular bundles remain intact whereas the surrounding softer tissues are dissolved. A network of hyphae is found throughout the collapsed tissues.

The first mention of brown patch as a disease of lawns in general was by Taylor (5). However, he did not determine the incitant. Piper and Coe (4) later reported that Rhizoctonia solani was the pathogen responsible for brown patch of lawn and pasture grasses near Washington, D. C. Their attempts to control the disease with fungicides were unsuccessful. Carrier (2) reported that light dustings with powdered Bordeaux mixture were of some value in combating brown patch. The fungicide in most cases did not eliminate the fungus but was able to prevent its spread to surrounding healthy turf. Monteith and Dahl (3) in 1932 recommended the use of a 50/50 bichloride of mercury/calomel mixture for the control of brown patch. This mixture remained the standard fungicide for the control of turf diseases until recently.

PROCEDURE

Isolations

Specimens of St. Augustine grass showing typical symptoms of brown patch were collected from a large number of lawns in several localities in Louisiana. Isolations were made from surface-sterilized portions of diseased sheaths and culms. Rhizoctonia solani was almost invariably isolated from such material. Other fungi often isolated were Fusarium sp. and an

FIGURE 1. St. Augustine grass lawn showing typical attack of brown patch.

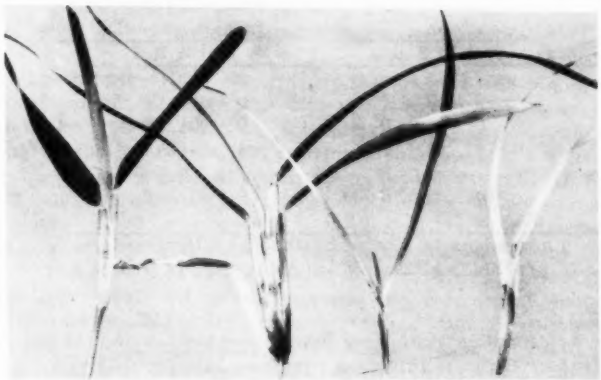

FIGURE 2. Comparison of healthy and brown-patch affected sprigs of St. Augustine grass. Healthy sprig on the left with three diseased sprigs on the right.

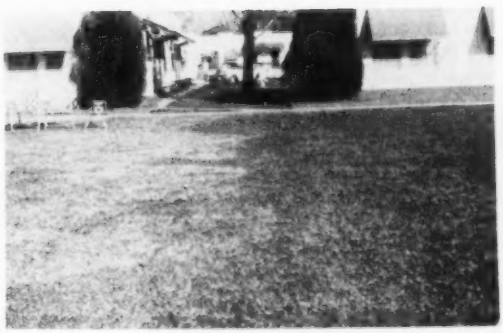

FIGURE 3. Chemical control of brown patch. Area on the right treated with Terraclor at the rate of 20 g/100 square feet. Area on the left untreated.

unidentified sterile white fungus. Curvularia sp. was also occasionally isolated.

Inoculations

Inoculum was prepared by growing the respective fungi on moistened autoclaved oats in 500 ml flasks. Most of the inoculations were made on St. Augustine grass growing in flats 2 feet x 2 feet, but some were made directly on an open lawn partially shaded by trees. For the flats turf was taken from a healthy vigorously growing lawn known to be free from brown patch. Some of the flats were kept in a lath house (approximately 50 per cent shade) and others in direct sun. Inoculations were made by placing 200 g of the inoculum on the surface of the grass in each flat or on a 1 x 2 foot area on the open lawn, working it down into the turf to soil level, and watering it. The results of the first series of inoculations are presented in Table 1.

Table 1. Results of inoculations of St. Augustine grass with three different fungi under different environmental conditions.

	Disease index[a]								
	Flats in 50% shade			Flats in direct sun			Partially shaded lawn		
Fungus	R 1	R 2	R 3	R 1	R 2	R 3	R 1	R 2	R 3
Fusarium sp.				0	0	0			
White fungus	0	0	0	0	0	0	0	0	0
Rhizoctonia solani	3	3	4	4	4	3	4	4	4
Control	0	0	0	0	0	0	0	0	0

[a] 0 = no disease; 1 = browning of bases and edges of a few sheaths; 2 = killing of isolated sheaths, general unthriftiness of grass; 3 = killing of small patches of grass; 4 = killing of large areas of grass.

The results of these inoculations indicate rather definitely that Rhizoctonia solani is the cause of brown patch of St. Augustine grass. Definite infection resulting in typical brown patch symptoms was obtained in every case in which Rhizoctonia inoculum was used, and no disease developed either in the non-inoculated checks or in the plots inoculated with Fusarium sp. or with the sterile white fungus.

A second series of inoculations was set up with two objectives: 1) to get some information on the influence of temperature on the development of the disease, and 2) to determine whether or not the Rhizoctonia associated with brown patch is a specialized race of R. solani particularly pathogenic to St. Augustine grass. Since the incidence of the disease coincides with the cool season of the year (early fall to late spring) it was thought that the high summer temperature might be the factor responsible for checking the development of the disease during the summer months. Also, since R. solani is a universal soil inhabitant and unquestionably present in all lawns but, on the other hand, the incidence of the disease is sporadic, it was thought that perhaps a specialized race of the fungus particularly pathogenic to St. Augustine grass might be responsible and that the disease occurred on this grass only when this race was present in a particular locality.

St. Augustine grass growing in flats 2 feet x 2 feet was inoculated with isolates of Rhizoctonia from the following hosts: St. Augustine grass, Bermuda grass, begonia, potato, and strawberry. The flats were placed in constant temperature rooms at 60°, 70°, 80°, and 90° F. Light was provided by banks of fluorescent tubes for 12 hours a day. The results of this test are shown in Table 2.

Temperature had a decided effect on the rate of development of disease. A rating of 4 (complete killing of large areas of grass) was attained in 10 days at 90° F, in 21 days at 80°, and in 37 days at 70°. In the flats kept at 60° no disease developed in 50 days, at which time the experiment was terminated. The results of this controlled temperature experiment are at variance with the behavior of the disease in nature. No satisfactory explanation for this discrepancy can be offered at present. The results of the outdoor inoculations, on the other hand, agree closely with the behavior of natural infections. In most of the outdoor inoculations made in summer, disease did not develop until the advent of cool weather in the fall.

The results give some indication that the Rhizoctonia affecting St. Augustine grass may

Table 2. The results of inoculation of St. Augustine grass in flats with different isolates of Rhizoctonia solani at controlled temperatures.

Isolate	Disease index[a] at indicated temperature										
	60° F		70° F		80° F				90° F		
	R1	R2	R1	R2	R1	R2	R3	R4	R1	R2	R3
St. Augustine grass	0	0	4	-	4	4	-	-	4	-	-
Bermuda grass	0	-	4	4	3	4	-	-	3	4	-
Begonia	0	-	3	3	-	-	-	-	2	2	-
Potato	0	-	3	4	4	-	-	-	4	4	-
Strawberry	0	-	-	-	4	-	-	-	4	3	-
Control	0	0	0	0	0	0	2*	4*	0	0	0

[a] 0 = no disease; 1 = browning of bases and edges of a few sheaths; 2 = killing of isolated sheaths, general unthriftiness of grass; 3 = killing of small patches of grass; 4 = killing of large areas of grass.
*R. solani isolated from the diseased grass.

not be a specialized race, since the disease was also produced in this grass by isolates from the other hosts. It is true that some of these (from begonia in particular) caused less disease than the isolates from St. Augustine grass, but this was only relatively so. Also, it may be argued that since disease developed in some of the non-inoculated check flats and since Rhizoctonia was reisolated from these, the infection in the flats inoculated with the pure culture inoculum of isolates from other host plants did not come from this inoculum but from the Rhizoctonia naturally occurring in the soil. The fact that the sod used in this test was cut from a lawn that appeared healthy is no assurance that it was free from Rhizoctonia.

The Bermuda grass isolate in culture was distinctly different from all other isolates and could be identified by macroscopic comparison. Reisolation of the Bermuda grass isolate from diseased St. Augustine grass inoculated with it indicated that, in this particular case at least, Rhizoctonia from another host could infect St. Augustine grass and cause brown patch. At best, the results of this experiment can be considered as an indication and not as conclusive proof that the Rhizoctonia causing brown patch of St. Augustine grass is not a specialized race.

CONTROL

Several fungicides were screened in attempts to control brown patch. The studies were carried out on naturally infected lawns in the New Orleans and Baton Rouge areas over a 2-year period. The fungicides were measured out and applied so that an entire area of a lawn, part of which was diseased, was treated, not just the diseased portion. For example, on a 1000 square foot lawn where only 200 square feet of turf was diseased, the entire 1000 square feet of lawn was treated. The fungicide in this case would be measured out to cover 1000 square feet. The concentration of all fungicides is expressed in terms of commercial formulation and not in terms of active ingredients. The fungicides were applied in two ways: with a John Bean Model 33E 50-gallon sprayer maintained at approximately 200 pounds pressure, equipped with two T-jet 8006 nozzles with spray cones covering a swath 2 feet wide; or with a 3-gallon watering can with an ordinary rose. The fungicides were constantly agitated while being applied. The following fungicides were tested. The results of all tests are summarized in Table 3.

1. Cadminate -- cadmium succinate 60 per cent, cadmium 20 per cent.
2. Caloclor -- mercurous chloride 60 per cent, mercuric chloride 30 per cent, total mercury as mercury 73 per cent.
3. Crag Turf Fungicide 531 -- 95 per cent complex of cadmium calcium copper zinc sulfate-chromate; copper 9.9 per cent, cadmium 4.2 per cent, zinc 4.1 per cent, chromium 3.2 per cent.
4. Kromad -- tetramethylthiuram disulfide 16 per cent, cadmium sebacate 5 per cent, potassium chromate 5 per cent, malachite green 1 per cent, auramine 0.5 per cent.
5. Puratized 177 -- phenyl amino cadmium dilactate 100 per cent.
6. Puratized Agricultural Spray -- phenyl mercuri triethanol ammonium

Table 3. Summary of the results of tests with nine different fungicides for control of brown patch of St. Augustine grass.

Fungicide	Area treated (sq. ft.)	Rate/100 sq. ft. per application	Control
Cadminate	4000	1.4 g	-[a]
	400	7 g	-
Caloclor	750	12 g	-
	500	9 g	-
	500	9 g	-
Crag Turf Fungicide 531	100	10 g	-
	1500	10 g (2 appl. 60 days apart)	-
	1000	13 g	-
	2000	30 g	-
	500	91 g	-
Kromad	100	10 g	-
	1500	10 g (2 appl. 60 days apart)	-
	1000	15 g	-
	1000	15 g	-
Puratized 177	100	10 g	+
	1500	10 g (2 appl. 60 days apart)	+
Puratized Agricultural Spray	450	50 ml	+
	1000	48 ml	-[b]
	1000	48 ml	-[b]
	1000	48 ml	-[b]
Semesan Turf Fungicide	100	10 g	-
	1500	10 g (2 appl. 60 days apart)	-
	1000	15 g	-
	500	20 g	-
	500	40 g	-
	500	60 g	-
Tersan	100	10 g	-
	1500	10 g (2 appl. 60 days apart)	-
Terraclor	1500	10 g (2 appl. 60 days apart)	+
	2000	15 g (2 appl. 35 days apart)	+
	1000	15 g	+
	1600	24 g (2 appl. 21 days apart)	+
	1100	30 g (2 appl. 21 days apart)	+
	1000	35 g	+
	500	40 g	+
	750	28 g	+
	1000	55 g (2 appl. 7 day apart)	+
	1000	20 g	+
	500	30 g	+
	750	28 g	+
	500	54 g (3 appl. 7 days apart)	+[b]
	500	25 g	+
	800	58 g (2 appl. 7 days apart)	+[b]
	500	136 g	+[b]
	1000	91 g	+
	500	91 g	+
	500	91 g	+
	1500	60 g	+
	500	91 g	+
	200	113 g	+
	100	113 g	+
	100	113 g	+
	1000	91 g	+
	500	91 g	+
	500	91 g	+
	1000	45 g	+
	200	50 g	+
	1000	45 g	+
	950	47 g	+

[a] - = No control; + = control.

[b] Phytotoxic: Terraclor, at rates of 116 g/100 sq. ft. and above, caused the grass to turn from green to a brilliant red. This red discoloration disappeared at the end of 2 to 3 weeks. Puratized Agricultural Spray, when first used in New Orleans during the spring of 1957, controlled the disease and caused no injury to the grass, but when applied at similar rates the next fall in Baton Rouge, it did not control the disease, and caused burning of the grass.

lactate 7.5 per cent.

7. Semesan Turf Fungicide -- hydroxymercurichlorophenol 25.3 per cent.
8. Tersan -- tetramethylthiuram disulfide 75 per cent.
9. Terraclor -- pentachloronitrobenzene 75 per cent wettable powder.

It will be noted from the summary of fungicide tests (Table 3) that the evaluation of the effectiveness of the fungicide was made on a strictly practical basis. A rigid yardstick was used; a fungicide either was effective or was not effective. No attempt was made to measure shades or degrees of effectiveness. A fungicide was considered effective if it stopped the spread of the infection so that the healthy grass would grow back and reestablish itself on the killed areas, and ineffective if it failed to accomplish this.

Of the fungicides tested, Terraclor proved outstandingly effective. Because of its good performance this chemical was tested extensively (31 separate tests) over a 2-year period in order to determine the minimum effective dosage and the maximum dosage that the grass would tolerate without injury. It proved effective at as low a rate as 20 g per 100 square feet (Fig. 3) and safe at as high a rate as about 113 g per 100 square feet. At dosages slightly higher than 100 g per 100 square feet it exhibited a peculiar type of phytotoxicity. It did not burn the grass or stop its growth but turned it bright red. The red discoloration gradually disappeared in 2 to 3 weeks.

A second chemical, Puratized 177, gave good control at the rate of 10 g per 100 square feet in two separate tests. Unfortunately, our supply of this fungicide was inadvertently lost and it was not included in subsequent tests. This chemical definitely deserves further testing. Puratized Agricultural Spray gave inconsistent results. In one test in New Orleans in the spring of 1957 it gave good control and caused no injury at the rate of 50 ml per 100 square feet. However, similar dosage rates in tests in Baton Rouge in the fall of the same year failed to control the disease and caused burning of the grass.

None of the other fungicides gave satisfactory control of the disease at the rates tested. It was rather surprising that neither Caloclor nor Semesan, both turf fungicides of long standing, proved effective.

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APHID TRANSMISSION OF A VIRUS ASSOCIATED WITH SWEETPOTATO INTERNAL CORK AND MASKED IN FEATHERY-MOTTLE-INFECTED SWEETPOTATOES

E. M. Hildebrand[1] and F. F. Smith[2]

Summary

The virus associated with sweetpotato internal cork was transmitted from diseased to healthy Porto Rico plants by both aphid species tested (Myzus persicae (Sulz.) and Macrosiphum solanifolii (Ashm.)).

When the final results were recorded at the end of 13 and 1/2 months the aphids had transmitted a virus from sweetpotato infected with internal cork to sweetpotato in six different experiments. On the basis of root lesions their efficiency was less than 20 percent as was likewise true for graft transmission, but on the basis of foliage symptoms transmission was 100 percent positive.

The same aphid species were incapable of transmitting feathery mottle virus, but were able to pick up and transmit the virus associated with internal cork symptoms when it occurred as a masked contaminant in a sweetpotato clone from Russia (P. I. 105945) affected with feathery mottle. In these experiments aphids transmitted the virus contaminant from sweetpotato to sweetpotato about as readily as from plants in which it was present alone. The fact that no roots were symptomed at the end of the experiment can possibly be accounted for by the very low initial concentration of the cork virus, as demonstrated by indexing tests on Scarlett O'Hara morning glory, when in combination with feathery mottle virus.

Aphids readily transmitted the virus of the internal cork syndrome from sweetpotato and certain sweetpotato relatives to Scarlett O'Hara morning glory (two experiments) with an incubation period of usually less than 2 weeks (6 to 15 days). When the virus source was feathery-mottle-infected plants, the incubation period was usually 12 days or longer compared with about half that period (6 to 8 days) for plants affected with internal cork alone.

INTRODUCTION

In March 1956 Hildebrand and Smith (6) reported that the aphids Myzus persicae (Sulz.) and Macrosiphum solanifolii (Ashm.) transmitted a virus of the sweetpotato internal cork syndrome under greenhouse conditions. Herewith are presented detailed results of some of the experiments including the negative results for aphid transmission of feathery mottle virus.

MATERIALS AND METHODS

The first graft and aphid transmission experiments with the virus associated with internal cork required 13 1/2 months for completion because the criterion of transmission in sweetpotato was development of necrotic lesions in the roots. The inoculated plants were grown in 6-inch pots about 6 months or until enlarged roots had been produced. Because slicing one or more of the larger roots at harvest never revealed a single root lesion, the harvested roots were incubated 6 months in warm (75° F), high-humidity (90 percent relative humidity) storage to induce root symptoms. Subsequently these roots were bedded in sand 3 to 5 weeks for sprout production, and after sprouts were removed for planting the roots were sliced for determining presence or absence of brown to black spot lesions characteristic of internal cork. Because

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some of the roots shriveled, it was not always possible to carry all the roots through the 6-month storage period.

The virus transmission procedures with aphids were carried out in a greenhouse insectary at temperatures near 70° F and involved the following steps: The aphids were reared on cabbage in a cool greenhouse (60°). The desired aphid population was removed from cabbage and starved in ventilated dishes for 1 to 2 hours and then fed on pieces cut from infected sweetpotato foliage. When the leaf pieces were placed in the dishes, they were quickly populated by the aphids. At the end of 10 minutes diseased leaf pieces with 15 or more aphids were placed on healthy plants either by leaning the pieces against the stem base or by placing them on a slightly larger square of white paper on the youngest tip leaf to prevent direct contact. The transplanted aphids soon left the diseased leaf pieces and fed on the healthy indicator plants. After a set feeding period (about 2 hours) the aphids were destroyed by dipping the plants in a nicotine-soap emulsion. Then the plants were transferred to an insect-free greenhouse bench for incubation and symptom production. About 1 month after inoculation the plants were transplanted into 6-inch pots.

For comparison and as a check on methods of transmission, ten graft-inoculated Porto Rico plants were included in the first aphid-transmission experiment. Tip grafts from terminals of plants with chlorotic foliage symptoms were used as inoculum. Foliage from the same shoot was used for aphid inoculum.

The Porto Rico sweetpotato was adopted as the test plant because it is highly susceptible to the root-symptom phase of the internal cork virus syndrome. However, the root-symptom phase is not a reliable criterion for susceptibility to infection until after 6 months' storage at 75° F and 90 percent relative humidity.

In these experiments the NH-10 series of healthy Porto Rico test plants, originally received from North Carolina by S. P. Doolittle, were used exclusively. Grown in 4-inch pots containing moderately rich potting soil, the rooted cuttings or sprouts were ready for the aphid-transmission tests when in the five-leaf stage.

Upon harvest the roots were dried and cured 7 to 10 days on the greenhouse bench before they were placed in paper sacks for storage. Since the plants were potbound the greenhouse-grown roots were generally small and variable in shape and size and occasionally there was only one per plant.

When the 6-month storage period was completed, but before the roots were sliced, usually one root from each of the lots (original plants) was bedded in sand for sprout production. The first five or ten sprouts from each lot were potted in fertile soil and observed for chlorotic leaf spots by the "flush of growth" technique (4).

The inoculation phase of these studies on aphid transmission of the viruses associated with sweetpotato was conducted from December 1954 to April 1955. The inoculum consisted of symptomed sweetpotato foliage from two sources, Porto Rico having chlorotic leaf spots or ring spots of the internal cork syndrome alone and Russian seedling (P. I. 105945) with feathery mottle virus, symptoms of which largely, if not completely, masked the presence of the internal cork contaminant. Later the virus of the internal cork syndrome was aphid-transmitted also from sweetpotato to Scarlett O'Hara morning glory.

When healthy indexing hosts are used for virus identification, they must be in vigorous growing condition when the inoculations are made so that infection and symptom expression will be uniform (1).

EXPERIMENTAL RESULTS

Aphid Transmission of Internal Cork Virus from Sweetpotato to Sweetpotato

The first aphid-transmission experiment was started December 14, 1954, and concluded January 30, 1956. The five subsequent experiments, started between February and April 1955, also were terminated on that date. Myzus persicae was used as a vector in the first five experiments. The graft-inoculation check series, involving top-grafting of a series of ten Porto Rico plants, was started at the same time. On the basis of internal cork root symptoms an average of less than one of the five plants in each insect-transmission experiment was positive compared with two in the series of ten plants in the graft-inoculated checks. Obviously lesions in roots produced at the end of the first growing season in the greenhouse are not a completely satisfactory criterion of transmission of internal cork virus.

However, on the basis of sprout-leaf symptoms, recorded on February 20, 1956, all the aphid-inoculated plants and all graft-inoculated check plants in all six experiments were positively infected. Thus, presence of chlorotic leaf spot virus lesions on the multiple sprouts from the roots, regardless of whether the roots were symptomless or symptomed at the end of the study, indicated that the virus was masked in the roots. Details on these six experiments follow.

Experiment 1: On the basis of internal cork root symptoms only one of five aphid-inoculated sweetpotato plants had positive root lesions compared with two of ten graft-inoculated check plants. On the basis of leaf symptoms on sprouts, all in both categories were infected.

Experiment 2: In the second experiment starting on February 18 and using ringspotted sweetpotato leaves as source of virus (as in Experiment 1) one of five plants was positively infected on the basis of root symptoms. Again on the basis of foliage symptoms all five plants were positively infected when subjected to the "flush of growth" technique (4) (F, 2/5; G, 5/5; H, 2/5; I, 3/5; and J, 4/5). The letter designates the root and the numerator of the fraction represents the number of sprouts that showed positive chlorotic spots out of a total of five represented by the denominator, at the end of 1 month of growth.

Experiment 3: In experiment 3, started on March 4, aphids again transmitted the internal cork virus from sweetpotato to sweetpotato. Only one plant (E) of the five aphid-inoculated was positive for root lesions, but the sprout record showed that all roots that survived were positively infected (B, 4/5; D, 3/5; and E, 3/5).

Experiment 4: From the aphid-transmission experiment of March 21, roots of only two of the five inoculated plants were available for the final sprouting and slicing. However, one root of plant 5 in the series showed positive root lesions, whereas positive leaf symptoms were present on the sprouts of both survivors (A, 4/5; E, 1/5).

Experiment 5: Started also March 21 with roots harvested on September 8, experiment 5 also demonstrated virus transmission by M. persicae from sweetpotato to sweetpotato. However, there were no root symptoms and roots of only two of the five plants survived storage for producing sprouts before slicing. The results were as follows: B, 3/5; E, 4/5.

Experiment 6: In experiment 6, started on April 1, the aphid Macrosiphum solanifolii was used; positive root symptoms were observed on only one (C) of the five aphid-inoculated plants, but the sprouts showed that all the surviving roots were positively infected (B, 4/5; C, 5/5; D, 3/5; E, 5/5). Thus this aphid species is as capable as Myzus persicae of transmitting the virus from sweetpotato to sweetpotato under greenhouse conditions.

Roots from non-inoculated plants of the test Porto Rico sweetpotato stock were stored and sliced for examination in the same way as the roots of variously inoculated lots. Neither roots nor leaf symptoms were found in these controls if they were protected against chance infection. The stock protected in the greenhouse always yielded negative results when indexed on Scarlett O'Hara morning glory.

Aphid Transmission of a Contaminant Virus from Feathery-Mottle Infected Sweetpotato to Healthy Sweetpotato

The following series of four experiments demonstrated that Myzus persicae is capable of picking up and transmitting the sweetpotato virus associated with internal cork when in mixture with and largely masked by feathery mottle virus in sweetpotato from Russia (P. I. 105945).

Experiment 1. Employing symptomed leaves of sweetpotato as inoculum experiment 1 (started December 14, 1954 and concluded January 31, 1956) explored the possibility of transmission of feathery mottle virus by Myzus persicae. Foliage symptoms indicated that feathery mottle virus was not transmitted, and no root symptoms of internal cork were evident. However, leaf symptoms of internal cork virus syndrome developed on sprouts of all four surviving sweetpotato plants (A, 6/8; B, 3/5; C, 3/5; and D, 3/5).

Experiment 2: Started on February 23 with the same source of feathery mottle inoculum, experiment 2 again demonstrated the inability of M. persicae to transmit feathery mottle virus. The sprouts showed that all surviving roots were infected with the associated virus (A, 2/5; B, 2/5; C, 1/5; D, 3/5), but no symptoms of internal cork were produced in sweetpotato roots.

Experiment 3: Experiment 3, using Myzus persicae but another source plant of P. I. 105945 affected with feathery mottle virus, was started on March 23. The aphid-inoculated sweetpotato plants developed neither symptoms of feathery mottle nor internal cork root lesions, but leaf symptoms on sprouts showed that roots of all four surviving plants were infected with the virus associated with sweetpotato cork (A, 1/5; B, 2/5; C, 1/5; and D, 3/5).

Experiment 4: In experiment 4, started April 1, Macrosiphum solanifolii was used, but the same contaminated source of feathery mottle virus as in experiments 1 and 2 was used as inoculum. The results were the same as before. No root symptoms developed, but the appearance of leaf symptoms in a considerable percentage of the sprouts from each of the surviving roots indicated infection with the virus associated with internal cork (A, 3/5; B, 4/5; C, 2/5; D, 2/5; and E, 3/5).

The total lack of root symptoms in plants infected in these experiments can possibly be accounted for by the very low initial concentration of the virus of the internal cork syndrome in coexistence with feathery mottle virus.

Aphid Transmission of the Sweetpotato Virus Associated with Internal Cork of Sweetpotato to Scarlett O'Hara Morning Glory

The C Series of experiments was initiated on May 9 and concluded on June 18, 1955. Previously it had been shown by using Scarlett O'Hara as an indicator plant that the virus associated with internal cork was mechanically transmissible (2).

Experiment 1: Experiment 1 tested transmission of the internal cork virus syndrome from plant to plant employing Scarlett O'Hara morning glory. Aphid transmission experiments using M. persicae were started on May 9 and concluded 18 days later, when results on all five inoculated plants were positive. Positive vein-banding mottle was present on plants B and E at the end of 11 days, on A and C at the end of 15 days, and on D on the eighteenth day. In this experiment the test plants were unpruned and two of the first leaves were approaching mature size when aphid-inoculated. In a parallel mechanical-transmission experiment with leaves of diseased sweetpotato and Scarlett O'Hara morning glory as inoculum, 100 percent positive results were recorded on the fifteenth day.

Experiment 2: Starting on June 11, spot-mottled leaves of Pharbitis diversifolia were used as inoculum (Fig. 1), and again 100 percent transmission to Scarlett O'Hara morning glory by M. persicae was obtained by the end of the twelfth day. A parallel experiment attempting aphid transmission of the same virus from P. diversifolia to black- and white-seeded moonflower (Calonyction aculeatum) gave negative results.

DISCUSSION AND CONCLUSIONS

For the internal cork virus of sweetpotato to build up and become widespread in the United States within a decade after its first published description (3) was strong evidence of natural transmission, presumably by insects as well as by mother roots.

The observation was made early in 1955 that discrete chlorotic spots are induced in leaves of infected sweetpotato plants put through a spurt of growth whether or not root symptoms were showing. This observation was followed up during the succeeding years in both the greenhouse and the field and confirmed in detail (4). It appears that Porto Rico sweetpotato plants are prone to give symptom expression in response to the "spurt of growth" shortly after transplanting the sprouts.

Up to 1955 only one positive criterion for internal cork of sweetpotatoes was known, namely, terminal necrotic spots in the roots, and expression of this symptom often required a year or longer, including 6 months' storage under the most favorable incubation conditions (75° F and 90 percent relative humidity).

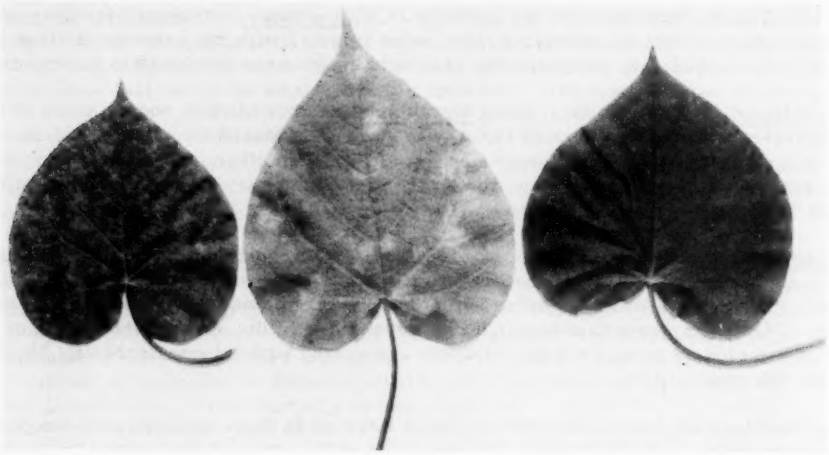

FIGURE 1. Pharbitis diversifolia (morning glory) one of the sources employed for the so-called internal cork virus, showing two kinds of chlorotic spots and the symptomless check.

In this investigation on aphid transmission, root lesions were present in less than 20 percent of the aphid-inoculated Porto Rico plants at the end of one growing season. Moreover, the lesions were small and few. The ten graft-inoculated Porto Rico check plants gave practically identical results, less than 20 percent of the roots having tiny cork lesions. Obviously these results appear out of line with what happens to sweetpotato roots of many plants grown in the field, but recent unpublished studies of root symptom development in plants inoculated in the field confirm these results.

However, the 100-percent transmission, based on the foliage symptom criterion, suggests the possibility of a single virus in the sweetpotato cork syndrome.

Unpublished studies on incubation period and seasonal development indicate that random multiple inoculation with virus in the field could account for the prevalent wide range of root symptom expression from a trace to very severe symptoms. Another possibility is resistant strains of sweetpotato. Under controlled greenhouse conditions only a single inoculation occurred and only a trace of root symptoms developed. When, in later experiments, the storage period was extended from 6 to 18 months, the percentage incidence of infections remained unchanged but disease severity (size of lesion) had increased somewhat.

Using the Scarlett O'Hara morning glory as an indexing host under controlled conditions has largely overcome the major obstacles to study of sweetpotato cork virus transmission. The rapid transmission technique (2) indicates that a single virus is involved in the internal cork syndrome and that this virus is readily transmitted mechanically and by aphids. When this indicator plant is employed, any symptoms appearing in the greenhouse or field can now be verified for presence of virus within 10 days.

The "flush of growth" technique (4) reveals the presence of virus in all susceptible sweetpotatoes at any time after harvest, regardless of variety or length of storage period. At one extreme is the Porto Rico variety, the standard test plant, for expressing root and foliage symptoms of internal cork. At the other extreme is the sweetpotato from Russia (P. I. 105945), which shows mild foliage symptoms sparingly but completely masks root symptoms. When sweetpotatoes are infected with feathery mottle virus foliage symptoms of internal cork are largely masked.

On the basis of these studies the aphids Myzus persicae and Macrosiphum solanifolii are efficient vectors of the leaf-mottling phase associated with internal cork virus under greenhouse conditions. The spread of this virus in nature was reported earlier (5).

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SICKLE LEAF OF OLIVE

H. E. Thomas[1]

A disease of olive, called sickle leaf because of the laterally curved shape of affected leaves, is rather common in commercial plantings and elsewhere in at least four counties of California. From published accounts[2], correspondence, and conversations, there is evidence that the same disease occurs in Chile, Italy, and Portugal.

In addition to the curved shape, the leaf blade is often chlorotic, more often in the inner side of the curve (Fig. 1). Affected branches may be considerably stunted and the amount of fruit reduced.

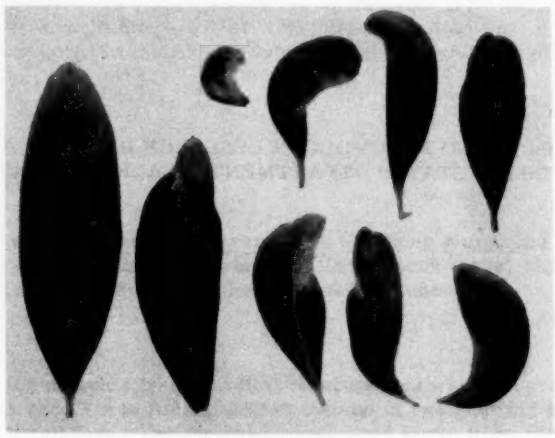

FIGURE 1. Sickle leaf of Mission olive produced by graft inoculation.

The disease appears to be systemic in individual branches but no tree, young or old, has been seen with all the branches clearly affected. For example, a tree grown from an affected cutting had many more "healthy" than visibly affected leaves and branches after 14 years.

Definite records of occurrence in the State seem to be limited to the "Mission" variety or group of strains widely grown in California.

Cions from healthy Mission olive on a diseased tree began to show symptoms in 17 months. Olive trees supplied by Dr. H. T. Hartmann under the names Manzanillo Sharpe, Mission Nobs, Oblonga, and Sevillano C. were inoculated by inarching to infected branches. The last named of these developed symptoms near the point of inoculation in 23 months, the Mission Nobs in 3 years, and the other two were still free of symptoms after 4 years. Single inoculations of oleander (Nerium oleander) and privet (Ligustrum sp.) did not produce symptoms in more than 3 years.

Since the presumed virus moves and develops extremely slowly in the tree, it does not seem likely to become of major importance in olive culture.

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[2] Ciferri, R., E. Baldacci, D. Rui, G. Scaramuzzi, G. Fogliani, and G. Rostirolla. 1953. Anomalie fogliari dell'olivo Ligure e Gardesano. Ann. Sper. Agr., NS7, 6, pp. 1957-1976, 12 figs. Original not seen. The sickle leaf in Italy was formerly associated with leptonecrosis but the latter is now attributed to boron deficiency (Ibid. 10, 1, pp. 25-59; 2, pp. 335-356, 25 figs., 1956), and the former to scale insects by some workers (Ciferri, Raffaele. 1956. Recent progress in fruit tree virus research in Italy. Tejdschrift over Plantenziekten 62: 69-72).

BEAN POD MOTTLE VIRUS OF SOYBEANS[1]

C. B. Skotland

Abstract

Pod mottle virus was found in naturally infected soybeans in North Carolina and Virginia. All soybean varieties included in inoculation tests were susceptible. The virus produced local lesions or mottling on various bean varieties. Some varieties of cowpeas were resistant and others were susceptible to infection. Annual and perennial lespedezas, velvet bean and crimson clover are reported as hosts for the first time. Lima bean, pea, red clover, sweet clover, crotalaria, broadbean, peanut, alfalfa, ladino clover, tobacco, cucumber, pepper, tomato and petunia were resistant. The virus was not transmitted by soybean seeds.

Disease surveys were made on soybean (Glycine max (L.) Merr.) in 1955 in eastern North Carolina and southeastern Virginia. Four samples of a soybean virus disease were collected from North Carolina and one from Virginia. In one field 75 percent of the plants were infected with the virus. A similarly diseased field of soybeans was also observed near Plymouth, North Carolina in 1954. Several varieties of soybean, bean (Phaseolus vulgaris L.) and other plant species were inoculated with these virus collections. These inoculations were made in the greenhouse at temperatures ranging from 75° to 85° F. Beans were inoculated by conventional mechanical methods when the primary leaves were almost expanded. Other hosts were inoculated in the 4- to 6-leaf stage. At least 15 plants of all species and varieties were inoculated. Plants which showed no visible symptoms were checked for the presence of the virus by inoculation to Idaho Refugee bean, a local lesion host for the pod mottle virus.

Plants inoculated with each of these four viruses had identical host ranges and symptoms which resembled the pod mottle virus described by Zaumeyer and Thomas[2]. The unidentified viruses and the pod mottle virus obtained from Dr. W. J. Zaumeyer of the United States Department of Agriculture, Beltsville, Maryland reacted identically on several hosts; however, the reaction of stringless Black Valentine snapbean[3] and Greenseeded Henderson Bush lima bean was not the same as that reported by Zaumeyer and Thomas for pod mottle virus. According to the original report, stringless Black Valentine was susceptible to local lesion infection only and in these tests it was systemically infected. Greenseeded Henderson Bush was reported to be a local lesion host but in these tests it was resistant to infection. A second isolate of the pod mottle virus was obtained from Zaumeyer and extreme caution was used to avoid contamination. This isolate also caused the same reaction on these bean varieties as those collected from soybeans. It was concluded that these soybean viruses were the pod mottle virus.

All soybean varieties tested were susceptible. These included S 100, Jackson, Ogden, Roanoke, Lee, Acadian, Palmetto, Laredo, Ralsoy, Illini, Richland, CNS, F.C. 33123[4], Mukden and Biloxi.

The cowpea (Vigna sinensis (Torner) Savi) varieties Black and Purple Hull were susceptible, however the variety Dixie Lee was resistant. Lespedeza stipulacea Maxim, var. Rowan, L. cuneata (Dumont) G. Don var. Sericea, L. striata (Thunb.) H. & A. var. Kobe, velvet bean (Stizolobium deeringianum Bort.), crimson clover (Trifolium incarnatum L.) var. Dixie Reseeding were also susceptible to systemic infection and are hosts not previously reported for this virus.

The following beans were susceptible to systemic infection: Cherokee Wax, Pencil Pod,

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

[2] Zaumeyer, W. J., and H. R. Thomas. 1948. Pod mottle, a virus disease of beans. Jour. Agr. Res. 77: 81-96.

[3] Seed was obtained from four different seed companies.

[4] Selection from Woods Yellow. Number given to it by United States Department of Agriculture.

Black Wax, Dwarf Horticultural, Stringless Black Valentine, Tendergreen, Burpee's Stringless Green Pod and Bountiful. Local lesions were produced on Pinto, Chervet, Wade, Kentucky Wonder, Idaho Refugee, Tendergreen, U. S. Refugee #5, Commodore, Contender and Ideal Market. Purple vein discoloration and purple local lesions on inoculated primary leaves, but no systemic infection, were noted on Blue Lake, Michelite, Navy and Keeney Refugee.

Non-susceptible legumes included lima bean (P. lunatus L.) var. Fordhook and Green-seeded Henderson Bush; sweet clover (Melilotus alba Desr.); pea (Pisum sativum L.) var. Alaska, Wisconsin Perfection and Perfected Wales; Crotalaria spectabilis Roth and C. mucronata Desv.; red clover, (T. pratense L.); broadbean (Vicia faba L.); peanut (Arachis hypogaea L.); alfalfa (Medicago sativa L.) var. Ranger, Atlantic, and Williamsburg and ladino clover (T. repens L.); tobacco (Nicotiana tabacum L.); cucumber (Cucumis sativus L.); pepper (Capsicum frutescens L.); tomato (Lycopersicon esculentum Mill.); and petunia (Petunia hybrida Vilm.).

The pod mottle virus produced a chlorotic mottle on all soybeans tested, and plants of F. C. 33123 were severely stunted. Local lesions were produced on some bean varieties and varying degrees of mottling on others. Occasionally the only symptom produced on Bountiful was chlorotic spots on the trifoliate leaves. Top necrosis was produced on velvet bean. Vein purpling and purple ring spotting followed by necrosis and chlorotic flecking were common symptoms on the cowpea varieties Black and Purple Hull. On the perennial lespedezas a slight chlorotic mottling was noted. On crimson clover and Kobe and Rowan lespedeza, the chlorosis produced tended to follow the veins but it spread also into the interveinal tissue.

Ralsoy, Ogden and Roanoke soybeans grown in the greenhouse were inoculated when primary leaves were expanded, with an isolate collected in North Carolina. Seed from these infected plants produced 605 plants, none of which showed symptoms of virus infection. Therefore it was assumed that the bean pod mottle virus is not seedborne in soybeans.

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INTERRELATIONSHIPS OF CERTAIN BETA SPECIES AND HETERODERA SCHACHTII, THE SUGAR-BEET NEMATODE

A. Morgan Golden[1]

Summary

In detailed studies conducted in the laboratory and greenhouse, root diffusate of each of three wild Beta species was found to be as effective as that of sugar beet in stimulating emergence of larvae from cysts of the sugar-beet nematode, indicating presence of a strong "hatching factor." Larvae of the sugar-beet nematode were found to enter the roots of the three wild beets and of sugar beet in large numbers; but mature female nematodes and cysts developed only on the roots of sugar beet. Some intermediate developmental stages and a very few mature males were observed on all three species of wild beets. A pathogenicity test failed to reveal a significant reduction in total plant weight of any of the three wild beets as a result of attack by the sugar-beet nematode. In the same test, however, sugar beet showed a highly significant weight reduction as compared with sugar beets in sterilized soil. The results of these studies are discussed particularly as they relate to the nature of resistance in these three wild Beta species.

Three wild species of the genus Beta (B. patellaris Moq., B. procumbens Chr. Sm., and B. webbiana Moq.) have certain characteristics which would be very desirable in the commercial sugar beet (B. vulgaris L.). Probably referring to some of these plants, Coons (2) and Doxtator (4) pointed out 20 years ago that certain wild species might serve as valuable sources for resistance to curly-top virus and Cercospora leaf spot. Stewart (11) noted that B. procumbens was reported by two German workers as resistant to the sugar-beet nematode, Heterodera schachtii Schmidt, and that this species, as well as B. patellaris and B. webbiana, has immunity to Cercospora leaf spot and at least high resistance to curly-top virus. Other workers, including Hijner (6) and Winslow (14), have found that all three of these Beta species are resistant to the sugar-beet nematode. Hijner (6) further reported that all these species produce a substance that is very effective in stimulating emergence of larvae from cysts of the sugar-beet nematode. Although these emerged larvae penetrated the roots of B. patellaris, they did not develop beyond the second larval stage. More recently, Shepherd (9) reported that about 7 percent of the sugar-beet nematode larvae that invaded the roots of B. patellaris developed to maturity as males, but she observed no mature females.

The present work was conducted with the broad objectives of obtaining a better understanding of the relationships existing between the sugar-beet nematode and all three wild Beta species (since these wild species may serve as excellent sources of resistance) and of obtaining information that might be of use to workers attempting to transfer resistance to commercial sugar beets. The nature of resistance in these wild beets is also of interest. Specific objectives of the present studies were 1) to determine the relative effectiveness of water and of root diffusates of sugar beet and of the three wild beet species on emergence of larvae from cysts of the sugar-beet nematode; 2) to ascertain how many larvae enter the roots of these plants and to study their development after entering the roots; and 3) to determine whether the larvae which enter the roots cause any reduction in overall plant growth, as indicated by total plant weight.

MATERIALS AND METHODS

Larval Emergence Test

Procedures and techniques used in the present test were essentially the same as those previously described by Golden (5) in conducting other larval emergence experiments. Briefly,

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fresh root diffusate was collected at weekly intervals from potted test plants (Beta patellaris, B. procumbens, B. webbiana, and sugar beet) which, after being used for 3 successive weeks, were discarded and replaced with young plants.

All tap water used was obtained locally from a deep well on the grounds of the United States Agricultural Research Station and was not subjected to any kind of chemical treatment, as is usual with city water.

The required number (800)of relatively young but mature cysts were obtained by selecting under a dissecting microscope uniform cysts from many which developed on sugar beets growing in soil heavily infested with cysts of the sugar-beet nematode. Cysts chosen for experimentation were kept in tap water in Syracuse watch glasses which were placed in a refrigerator for 10 days at approximately 1.1° C. During this time the tap water was drawn off daily with a pipette and immediately replaced.

Immediately after this period in the refrigerator, the 800 selected cysts were divided into five uniform groups so that the cysts in any one group were similar in size, shape, color and plumpness to those in the other groups. A group therefore consisted of 160 cysts and was subdivided into four replications of 40 cysts each. The 40 cysts of each replication were contained in a separate watch glass.

The experiment was conducted for 6 weeks in a dark, aerated cabinet in the laboratory at temperatures of approximately 25° C during the day and 20° at night. Fifteen ml of fresh tap water or the appropriate root diffusate were placed in each of the four watch glasses containing the 160 cysts of a particular group. The tap water and root diffusates were changed weekly and the emerged larvae were removed and counted.

Larval Penetration and Development Tests

To determine whether larvae entered the roots and to study their subsequent development, 28 young plants of each of the three wild Beta species, as well as 28 of sugar beet, were placed in 6-inch pots containing homogeneously mixed soil heavily infested with cysts of the sugar-beet nematode. These were grown in the greenhouse at a temperature ranging from not below 22.2° C to about 30° during the day. Every 15 days during the 60-day test period, seven plants of each of the four species were removed and examined. The plants were checked both macroscopically and microscopically for white female nematodes and cysts on the roots before and after the soil was gently removed from the roots. After being rinsed and weighed, the roots were stained in a boiling solution of lactophenol and acid fuchsin and then placed in a similar solution without the stain (7). Later, the stained roots of each individual plant were fragmented with water in a Waring Blendor; from this resulting mixture of water, nematodes and blended roots, the number of nematodes in the roots and their degree of development were determined by microscopic examination. As suggested by Doncaster (3), the mixture was not sieved and aliquots were easily taken when necessary for counting the nematodes.

Representative portions of soil from the pots containing the four test plants were processed by the sieve and Baermann funnel method (1) at each 15-day check period to recover H. schachtii males, which were counted, and the number per plant was calculated.

Pathogenicity Test

To determine whether the sugar-beet nematode reduced plant growth as indicated by total plant weight, 15 young plants each of B. patellaris, B. procumbens, B. webbiana and sugar beet were placed individually in 8-inch pots containing homogeneously mixed soil heavily infested with cysts of H. schachtii. At the same time, an equal number of similar plants were put individually in 8-inch pots containing sterilized soil. Each plant species was therefore represented by 15 plants (replications) in cyst-infested soil and a similar number in sterilized soil. All plants were grown for 3 months in the greenhouse under environmental conditions similar to those in the preceding test. Then the plants were harvested and the total weight of each plant with its root system was determined. During this procedure, observations were made for the presence or absence of white nematode females and cysts on the roots.

RESULTS AND DISCUSSION

The data obtained in these experiments are presented in Tables 1 to 4.

Table 1 shows that root diffusate from each of the three wild beets stimulated very many larvae to emerge from the cysts during the first week, an effect similar to that obtained with

Table 1. Weekly emergence of larvae of the sugar-beet nematode from cysts in tap water and root diffusates of certain Beta species during 6-week test period.

Time (weeks)	Test plants[a] and larvae emerged: B. patellaris	B. procumbens	B. webbiana	B. vulgaris (sugar beet)	Tap water
1	7932	8512	8480	7520	1549
2	4040	3664	3496	4056	1777
3	1088	773	1063	1292	1570
4	749	725	512	559	325
5	253	284	243	171	141
6	96	76	184	80	127
TOTAL	14, 158	14, 034	13, 978	13, 598	5489
Average larvae per cyst	88.5	87.7	87.4	85.0	34.3

[a] For each test plant, as well as for tap water, 160 cysts divided into four replications of 40 cysts were used, making a total of 800 cysts in the test.

Table 2. Entrance and development of H. schachtii larvae in roots of certain Beta species grown in cyst-infested soil for different periods of time.

Exposure periods and test plants	Average number of nematodes: Per plant	Per gram of root	Stage of development[a]
15-day			
B. patellaris	43	131	2
B. procumbens	77	119	2
B. webbiana	76	279	2; 3
B. vulgaris (sugar beet)	366	474	2; 3
30-day			
B. patellaris	1077	847	2; 3; 5M
B. procumbens	785	529	2
B. webbiana	1817	949	2; 3; 5M
B. vulgaris (sugar beet)	2614	514	2; 3; 4; 5M; 5F
45-day			
B. patellaris	1410	134	2; 3; 5M
B. procumbens	963	85	2; 3; 5M
B. webbiana	1560	118	2; 3; 5M
B. vulgaris (sugar beet)	2505	149	2; 3; 4; 5M; 5F; C
Grand averages			Number of adults per plant
B. patellaris	843	371	M-18.2; F-0
B. procumbens	575	244	M-0.3; F-0
B. webbiana	1151	448	M-11.6; F-0
B. vulgaris (sugar beet)	1828	379	Many M, F and C

[a] 2, second-stage (infective) larvae; 5, mature specimens; M, males; F, females; C, cysts.

Table 3. Entrance and development of H. schachtii larvae in roots of certain Beta species grown in cyst-infested soil for 60 days.

Exposure period and test plants	Average number of nematodes: Per plant	Per gram of root	Stage of development[a]
60-day			
B. patellaris	703	58	2; 3; 5M
B. procumbens	361	14	2; 3; 5M*
B. webbiana	422	23	2; 3; 5M
B. vulgaris (sugar beet)	17,192	588	2; 3; 4; 5M; 5F; C

[a] 2, second-stage (infective) larvae; 5, mature specimens; M, males; F, females; C, cysts.
* only 0.3 male per plant.

root diffusate of sugar beet. In succeeding weeks, a progressively smaller but similar number of larvae emerged in each of the root diffusates, but even less emerged in tap water. The total number of emerged larvae and the average number of emerged larvae per cyst were about the same in each of the diffusates, indicating that the three wild beets, as well as sugar beet, produce a substance or substances stimulatory to emergence of larvae from cysts of the sugar-beet nematode.

Although the stimulatory effect upon larval emergence seems to be the same with all these diffusates, there is no evidence that the stimulatory material produced by these four plants is identical in all other characteristics. It is possible that the "hatching factor" from one or more of these wild Beta species is different in some of its chemical or physical properties from the one in sugar beet. Winslow and Ludwig (15), in extensive studies on stimulation of emergence of larvae from cysts of the sugar-beet nematode, suggested that the "hatching factor" produced by different plants may be somewhat different. In support of their contention, they reported that phenol extraction by the chromatographic procedure was ineffective in removing the "hatching factor" for H. schachtii from beet-root diffusate, but was effective in removing it for the same nematode from root diffusate of rape.

Results obtained in the larval penetration and development tests for the first 45 days (three exposure periods) are presented in Table 2 and data for the 60-day exposure to the nematode are given in Table 3. At the end of the 15-day exposure period, the average number of nematodes per gram of root in the wild beets was variable and lower than in sugar beet. After 30 days of exposure, however, there were large numbers of nematodes in the roots of all test plants, and relative numbers of nematodes per gram of root at this time and after the 45-day exposure period indicate that there is no morphological barrier to larval entry into the roots of these wild beets. Furthermore, there seems to be no evidence of effective repellency of the larvae by the roots as compared with those of sugar beet, or of failure of the roots to attract the larvae (10, 12, 13).

The development of the sugar-beet nematode larvae in the roots of sugar beet proceeded at a relatively rapid rate, approximating that described by Raski (8) for this species. Many swollen second-stage larvae as well as numerous third-stage larvae were observed upon examination of the roots after 15 days of exposure in cyst-infested soil. After 30 days of exposure many specimens in various stages of development, including mature males and gravid females, were found. After 45 days of exposure all stages of the nematode were found, including many brown cysts filled with eggs; after 60 days of exposure (Table 3) a second life cycle had already begun, as evidenced by large numbers of second-stage larvae in the roots, in addition to the various other stages.

On the other hand, development of this nematode on all three species of wild beet proceeded relatively slowly and only to a limited degree. No females were found on the roots of the wild beets at any time, even after the 60-day exposure period. At the end of 15 days of exposure in the cyst-infested soil little or no development of the larvae had occurred in the roots of B. procumbens or B. patellaris; only an occasional third-stage larva was found in the roots of B. webbiana. After 30 days of exposure no larval development was noted on B. procumbens, but some third-stage larvae, as well as a few mature males, were found on both B. patellaris

Table 4. Average total weight per plant of certain Beta species grown for 3 months in soil infested with cysts of the sugar-beet nematode.

Test plants[a]	Average total weight per plant (grams)	
	With nematodes	Without nematodes
B. patellaris	157.3	178.6
B. procumbens	174.7	150.1
B. webbiana	169.1	165.9
B. vulgaris (sugar beet)	47.7	192.2

[a] Each test plant was replicated 15 times with nematodes and 15 times without nematodes.

and B. webbiana. After both the 45- and the 60-day exposure period a limited number of third-stage larvae and mature males were found on all three wild beets. The average number of adult males found per plant (Table 2) would indicate that B. procumbens is less favorable for male development than are the other two wild beets.

Results of the pathogenicity test (Table 4) show that the weight of sugar-beet plants grown in sterilized soil was about four times that of plants grown in soil infested with cysts of the sugar-beet nematode. Statistical analysis of the data showed this difference to be very highly significant. With the three wild Beta species, on the other hand, there were no significant differences in weights of the plants grown in sterilized soil and those grown in similar cyst-infested soil as used with sugar beet above. Apparently the entrance and limited development of the larvae in the roots do not adversely affect the overall plant growth.

No female nematodes were observed on roots of the wild beets when the test was terminated at the end of 3 months.

Although the exact nature of the resistance of the wild beets is not known, the results of these experiments indicate that resistance to the sugar-beet nematode in the three wild beets is definitely not due to the following: 1) failure to produce an effective "hatching factor" to stimulate emergence of larvae from the protective cysts; 2) repellency of the larvae by the roots or a failure of the roots to attract the larvae; or 3) a morphological characteristic of the roots which prevents entrance of larvae into the roots. Rather, it would appear that resistance might be due to a factor, or factors, within the root (or plant) itself, possibly of a chemical or a physiochemical nature.

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UNUSUAL RESPONSE OF HESPERIS MATRONALIS L. TO ROOT-KNOT NEMATODES (MELOIDOGYNE SPP.)

A. Morgan Golden and Thelma Shafer[1]

Summary

In laboratory and greenhouse tests, Hesperis matronalis, a cruciferous plant which might be used as a trap plant for use in biological control of the sugar-beet nematode, was determined not to be a host for any of the four species and two subspecies of root-knot nematodes known to occur in the United States. Root-knot nematode larvae were found to enter the roots and cause formation of typical knots, but they did not develop to maturity. All species and subspecies of root-knot nematodes developed normally on tomato control plants, and all developmental stages were readily observed.

Hesperis matronalis L., a cruciferous plant, is among several plants which may offer some experimental possibilities as a trap plant for use in biological control of the sugar-beet nematode, Heterodera schachtii Schmidt. Unlike the susceptible trap plants which were used to some extent in Europe in the past (2, 3) and which had to be completely removed from the cyst-infested soil before the female nematodes reached maturity on the roots, H. matronalis can remain in the soil as long as necessary or practical because it is not a host for the sugar-beet nematode (6, 8). Even though a non-host, this plant was found by den Ouden (6) to produce a "hatching factor" as effective as that produced by sugar beet (Beta vulgaris L.) in stimulating emergence of larvae from cysts of the sugar-beet nematode. Den Ouden further reported that when grown in cyst-infested soil, both in pot tests in the greenhouse and in field experiments, this plant reduced the nematode population as determined by examination of cyst contents. In laboratory and greenhouse experiments during 1956, the senior author of the present paper also found that H. matronalis was as effective as sugar beet in stimulating emergence of larvae from cysts of the sugar-beet nematode and that larvae entered the roots in large numbers but subsequently developed very little, if any, and none reached maturity.

In the event that this or another such plant should prove to have practical value in the control of the sugar-beet nematode, it would be desirable to know its susceptibility to other important nematode parasites of sugar beets. The present work was initiated to determine the susceptibility of H. matronalis to the four species and two subspecies of root-knot nematodes (Meloidogyne spp.) known to occur in the United States.

MATERIALS AND METHODS

Nine young seedlings of H. matronalis and nine of tomato (Lycopersicon esculentum Mill.) were placed individually in 6-inch pots containing homogeneously mixed soil infested with either a species or subspecies of root-knot nematode. This procedure was repeated with each of the six root-knot nematodes being tested, as specified in Table 1. The plants were grown for 2 months in the greenhouse at temperatures ranging from 22.2° to about 30°C. Then they were removed from the pots and rated according to the amount of visible galling on the roots. Representative roots of both test plants were examined while fresh with the aid of a stereoscopic microscope, while other roots were stained in a boiling solution of lactophenol and acid fuchsin (5) and later examined microscopically also. Representative portions of soil from pots containing H. matronalis were processed by the sieve and Baermann funnel method (1) to recover males of each nematode species.

Results obtained in this first experiment suggested the need for a second test, in which 12 young seedlings of H. matronalis and 12 of tomato were potted individually as described above for each of the six root-knot nematodes. Half of these plants (six plants of H. matronalis and six of tomato for each nematode species and subspecies) were grown in the greenhouse as in the preceding test for 4 months before being harvested and rated for visible galling on the roots.

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Table 1. Root-knot rating[a] of Hesperis matronalis exposed to four species and two subspecies of root-knot nematodes (Meloidogyne spp.)

Nematode species and subspecies	Experiment 1[b]		Experiment 2[c]	
	H. matronalis	Tomato (control)	H. matronalis	Tomato (control)
M. arenaria	2.8	3.8	2.3	4.0
M. arenaria thamesi	0.9	4.0	1.0	4.0
M. hapla	0.6	3.3	3.0	4.0
M. incognita	1.1	4.0	2.6	4.0
M. incognita acrita	1.0	4.0	0.3	3.7
M. javanica	1.6	4.0	1.0	3.5

[a] Scale: 0, no infection (or galling); 1, trace; 2, light; 3, moderate; 4, heavy.
[b] Average of nine replications of each test plant for each nematode species or subspecies.
[c] Average of six replications of each test plant for each nematode species or subspecies.

Table 2. Entrance and development of larvae in roots of Hesperis matronalis during a 15-day exposure to four species and two subspecies of root-knot nematodes (Meloidogyne spp.)

Nematode species and subspecies	Average larvae per plant[a]		Nematode development[b]	
	H. matronalis	Tomato (control)	H. matronalis	Tomato (control)
M. arenaria	81.0	99.0	2, few 3	2, 3
M. arenaria thamesi	46.0	87.8	2, few 3	2, 3, 4
M. hapla	902.0	268.5	2	2, 3, 4
M. incognita	328.7	318.3	2, few 3	2, 3, 4
M. incognita acrita	28.0	40.5	2	2, 3
M. javanica	2.3	8.8	2	2, 3, 4

[a] Average of 6 replications of each test plant for each nematode species and subspecies.
[b] 2, second stage (infective) larvae; 3 and 4, intermediate stages; 5, mature specimens.

Roots of both kinds of test plants were further examined as previously, and representative portions of soil from pots containing H. matronalis were also checked for males. The other plants, after being grown in the greenhouse for 15 days, were harvested in order to determine whether larvae entered the roots of H. matronalis in appreciable numbers as compared with tomato and, if so, how much development occurred. Methods similar to those described by Golden (4) in studies on larval penetration and development of the sugar-beet nematode on certain species of Beta were used. The roots of each plant were rinsed in water, weighed, stained in lactophenol solution with acid fuchsin and later fragmented in a Waring Blendor to facilitate counting specimens and determining their degree of development with the aid of a stereoscopic microscope.

RESULTS AND DISCUSSION

The root-knot ratings obtained from the two experiments are presented in Table 1. In both tests, H. matronalis appeared susceptible to at least some degree to all species and subspecies of root-knot nematodes. Although the knots on the roots were usually smaller than those on tomato and also often had very small rootlets growing from them or nearby, they appeared to be reasonably typical of root-knot nematode infection (Fig. 1). However, no mature root-knot nematodes (males or females) were found during extensive microscopic examination of the roots of H. matronalis, even on the plants from the second experiment which was continued for 4 months. In both fresh and stained roots of plants from the first experiment, larvae in the second and sometimes the third stage of development were found in the knots, but usually in various states of disintegration. No larvae were found in the galled roots of the H. matronalis

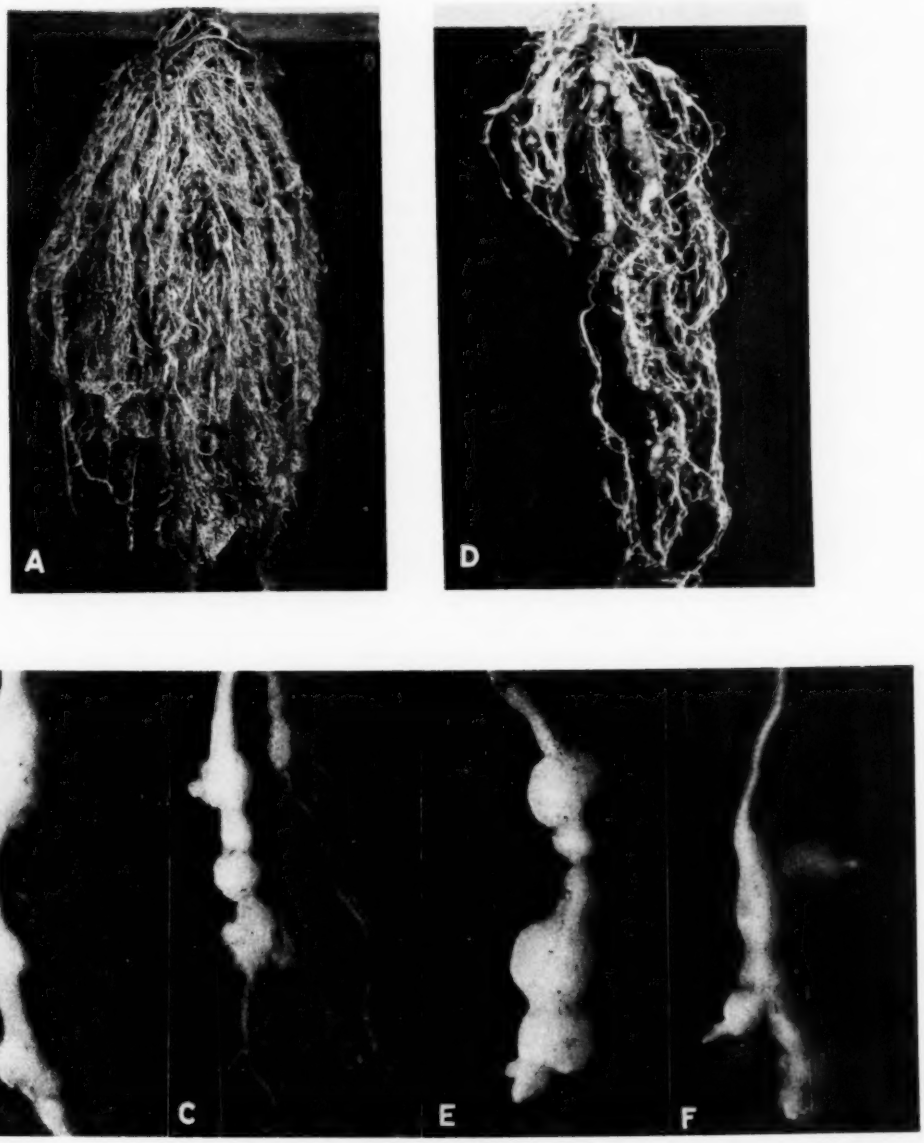

FIGURE 1. Roots of Hesperis matronalis and tomato attacked by Meloidogyne arenaria. A-C, H. matronalis. A -- Whole root system (about 12 inches in length). Note numerous small galls. B and C -- Photomicrographs of root segments with galls and small rootlets (diameter of galls about 2 to 3 mm). D-F, Tomato. D -- Whole root system (about 12 inches in length). E and F -- Photomicrographs of root segments with galls (diameter of galls about 2 to 3 mm).

plants from the second experiment, the larvae presumably having completely disintegrated during the 4-month test period, as would be expected in a non-host plant. No males were recovered from the soil in which the H. matronalis plants were grown, and galls were not observed on plants grown later in sterilized soil. In both tests all developmental stages of each of the nematode species and subspecies were found on tomato, the nematodes evidently having developed normally.

The data in Table 2 show that during the 15-day exposure to the root-knot nematodes in infested soil larvae entered the roots of H. matronalis in numbers generally comparable with those entering tomato roots but little, if any, development occurred. A majority of the larvae found within the H. matronalis roots were in the second stage but a few root-knot larvae of two species and one subspecies reached the third stage during this test period. Apparently little further development of the larvae within the roots of this plant would have occurred, as indicated in the preceding tests. On the other hand, larval development proceeded relatively rapidly in the roots of tomato, and many third-stage and even some fourth-stage larvae were observed.

These results indicate that H. matronalis is not a host for any of the four species and two subspecies of root-knot nematodes tested even though larvae entered the roots and produced typical symptoms. It seems that the root-knot rating of a previously untested plant should also be supplemented with microscopic examination of the roots because, as seen in the present case and as emphasized by Steiner (7), dependence upon macroscopic symptoms alone can be very misleading.

From a practical standpoint, it may be pointed out that H. matronalis would not only act as a trap plant for use in biological control of the sugar-beet nematode, but would also serve as a trap plant for root-knot nematodes.

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THE REACTION OF TRIFOLIUM SPECIES TO THE SOUTHERN ROOT-KNOT NEMATODE, MELOIDOGYNE INCOGNITA VAR. ACRITA. [1]

Norman E. McGlohon and L. W. Baxter[2]

Previous greenhouse investigations have revealed that all breeding clones of white clover (Trifolium repens L.) currently in use at Clemson are susceptible to the following root-knot nematodes: Meloidogyne javanica, M. arenaria, M. hapla, M. incognita, and M. incognita var. acrita. Among some 100 clones thus tested, apparent differences in susceptibility to root-knot were noted, but the degree of resistance did not appear adequate for the development of a white clover variety resistant to this disease. It therefore seemed advisable to test other Trifolium species as a possible source of resistance. Twenty-five species, representing both perennial and annual types, were used for this study.

Larvae of Meloidogyne incognita var. acrita, the southern root-knot nematode, were increased on Rutgers tomatoes and were used as inoculum.

Seedling plants, 18 plants of each species, were grown in 3-inch pots and were permitted to become thoroughly established prior to inoculation. At the time of inoculation, the pots were

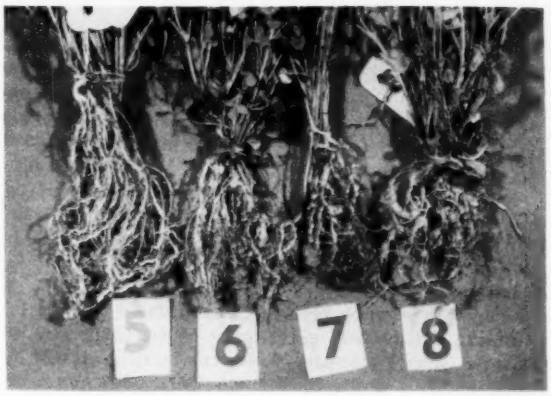

FIGURE 1. The reaction of 4 species of Trifolium to the southern root-knot nematode, Meloidogyne incognita var. acrita. 5 -- Trifolium amabile; 6 -- T. glomeratum; 7 -- T. angustifolium; and 8 -- T. radiosum.

FIGURE 2. The reaction of 4 species of Trifolium to the southern root-knot nematode, Meloidogyne incognita var. acrita. 17 -- Trifolium repens; 18 -- T. resupinatum; 19 -- T. tomentosum; and 20 -- T. pratense.

[1] Technical Contribution No. 292 of the South Carolina Agricultural Experiment Station, Clemson Agricultural College, Clemson, South Carolina.

[2] Research Assistant and Associate Plant Pathologist, respectively.

sunk in a sand-filled, electrically heated bench. An aliquot of inoculum was added to each pot and thoroughly mixed and watered into the soil. The temperature of the soil was maintained at 80°F for a period of 6 weeks, after which time each plant was indexed for relative susceptibility. The species tested were as follows:

Trifolium	Trifolium	Trifolium	Trifolium
alexandrinum	incarnatum	pallidum	scutatum
amabile	isthmocarpum	phleoides	spumosum
angustifolium	lappaceum	pratense	squarrosum
campestre	medium	radiosum	subterraneum
cherleri	michelianum	repens	tembense
glomeratum	nigrescens	resupinatum	tomentosum
hirtum			

All of the inoculated plants of all 25 species (15 to 18 plants of each species) were severely galled. Figures 1 and 2 illustrate the gall development on eight different species of Trifolium, which is comparable with development on the other 17 species. It thus appears most unlikely that root-knot resistance may be incorporated into white clover by hybridization with any of these species, unless a program of screening large numbers of seedlings or clonal lines should reveal resistant individuals.

During routine screening of white clover seedlings, it was noted that certain individual plants were not so severely galled as others. Only a very few seedlings of the several thousand tested showed resistance and the resistance in all instances was only partial. Thus, it is believed that a program of selection of superior white clover clones offers the best means of developing a variety of white clover resistant to this disease, unless some species other than those tested is found to have a high degree of resistance.

SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION,
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TESTING NEMATODE RESISTANCE IN THE FIELD[1, 2]

D. C. McGuire[3] and R. W. Allard[4]

Infestation by root-knot nematodes (Meloidogyne spp.) is rarely uniform throughout a field. Population densities also tend to vary from season to season and from year to year in local areas within the same field. Dependable tests of resistance to root-knot nematodes are therefore difficult to obtain in field trials. However, in three recent trials on the island of Oahu, Hawaii, large-scale testing of lima beans for pathological reaction to root-knot has been successful.

The first test, at the Poamoho Farm in the winter of 1956-1957 (November to March), included five commercial varieties, ten strains that had shown resistance in field and greenhouse trials in California[5], and 135 F_3 families derived from resistant strains crossed with susceptible commercial varieties. Table 1 shows the response of the test plants, with the reaction of F_3 lines indicated by a sample of four representative families.

Among the commercial varieties the only one showing any resistance was Westan, a variety developed by the California Agricultural Experiment Station. This variety develops occasional small to moderate galls in heavily infested fields in California, but is resistant enough to escape damage in commercial production. The test confirms its low to moderate resistance.

Since the ten strains bearing L prefixes were immune or nearly-immune in California greenhouse trials, their responses in field trials in Hawaii are of particular interest. Among these strains, all but L140 showed good resistance, and the resistance of L136 was outstanding (Fig. 1). Its roots were free of galls, and bright and smooth. L138 and L141 scored as high, but a few roots had tiny swellings and a roughened epidermis.

The four lines labelled H110 are representative F_3 families derived from Fordhook x L76. H110-40 is typical of a number of families which reacted similarly to the resistant parent. Subsequent progeny tests indicated that such lines were homozygous for resistance. Notice that H110-26 segregated more "cleanly" than did H110-24 or H110-29. Typical segregation is illustrated in Figure 2.

The second trial, also at the Poamoho Farm, was conducted in the summer (May to October, 1957). Most of the 66 entries were F_4 progenies derived from resistant plants selected in the first trial. Only two families were homozygous for susceptibility, indicating that the first trial was effective in identifying susceptible plants.

The third trial, at the Waimanalo Farm, was planted in November, 1957, and harvested in February, 1958. It included 106 entries, 45 of which were F_3 families previously tested repeatedly in greenhouse nematode trials in California. Among these 45 families, nine were homozygous for susceptibility, which indicates that the Hawaiian field tests are more effective in eliminating susceptible materials than the greenhouse tests.

DISCUSSION

The success of these field trials is attributed to three major factors. Most important appears to be the benevolent climate of the test areas. Temperatures varied only from 60° to 85° F, and minimum relative humidity was 55 percent. When necessary, rainfall was supplemented with irrigation. The resulting low moisture stress allowed nearly all plants to survive to maturity, even when the roots were nearly destroyed by heavy galling.

Second in importance was relative freedom from other pathogens. No diseases were observed in the first two tests. In the third, halo blight appeared during a cold, wet period, but dry weather and a tribasic copper sulfate spray stopped it before damage was significant. In the same test, about 1 percent of plants had symptoms resembling common bean mosaic. In all three trials insect pests were kept under control with spraying.

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[1] This work was supported in part by funds supplied by the California Lima Bean Advisory Board, Los Angeles, California.

[2] Published with the approval of the University of Hawaii Agricultural Experiment Station Director as Technical Paper No. 428.

[3] Associate Olericulturist, Hawaii Agricultural Experiment Station.

[4] Professor of Agronomy, University of California, Davis, California.

[5] Allard, R. W. 1954. Sources of root-knot nematode resistance in lima beans. Phytopathology 44: 1-44.

Table 1. Performance of lima bean stocks under attack by root-knot nematode.

Gall ratings:[a]	1	2	3	4	5
Variety	No. of plants				
Hendersons' Baby Bush					25
Burpee's Fordhook					25
Ventura					25
Wilbur	1				13
Westan		1	6	11	12
L76	3	15	4	1	
L133	4	21		1	
L134	19	4		1	
L135	19	6			
L136	26				
L137	5	6	4	8	2
L138	25		1[b]		
L139	2	12	11		
L140		6	3	6	10
L141	25				
H110-40	20	6	3		
H110-24	1	15	9	4	13
H110-26	7	19	1	1	7
H110-29	7	11	6	2	10

[a] Gall ratings are: 1-- free, or slight root roughening at most. 2-- a few small galls. 3-- several small or a few medium-sized galls. 4-- a few large galls. 5-- many large galls. These scores are relative since, even with care, portions of the roots were broken off while being lifted with the spading fork. Since clinging soil was readily brushed off, the roots were not washed.

[b] Atypical plant, probably resulting from an outcross.

The third factor favoring success was the relative uniformity of infestation by root-knot nematodes. Such variation as existed in the test area was compensated for by alternating test plants with morphologically distinctive susceptible controls within rows (4-inch spacing), or by planting test and check plants on opposite sides of the same bed. In either case, root symptoms of test and control plants were intermingled. A few lightly infected check plants were found in test 3 (Waimanalo). The adjacent test plants thus cannot be said to be resistant until they have been progeny tested. Figure 3 shows root systems of Ventura, a susceptible commercial type, and L76, a resistant type, under heavy and under light attack in test 3.

Correlation was excellent between the responses in California and those in Hawaii. The main difference was that infection was more uniform and severe under Hawaiian conditions. This suggests that the same species and physiological races of root-knot nematodes attack lima beans in both places. The primary organism attacking lima beans in both California and Hawaii is Meloidogyne incognita acrita. There is some evidence that other species of Meloidogyne cause roughening or insignificant galling of the roots of certain lima bean genotypes[6].

[6] The authors are indebted to Dr. O. √. Holtzmann, Pathologist, Hawaii Agricultural Experiment Station, and Dr. D. J. Raski, Nematologist, University of California for their assistance.

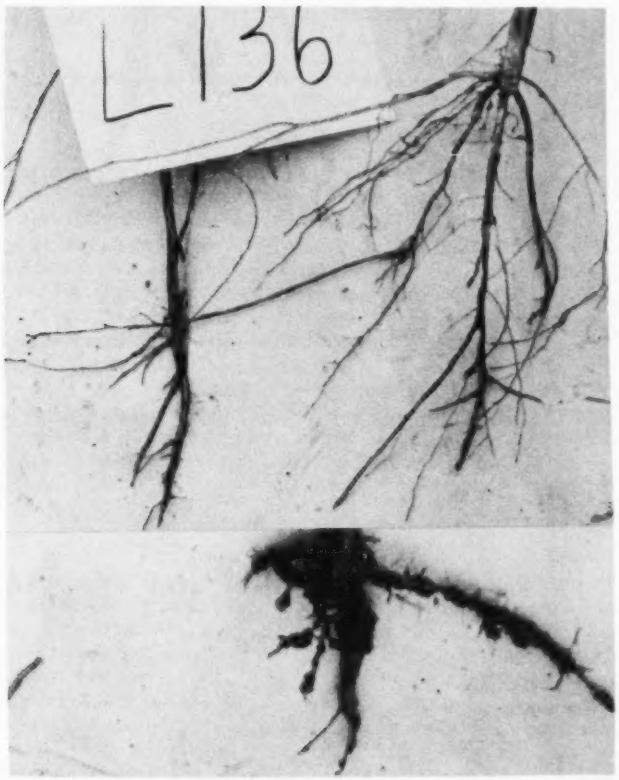

FIGURE 1. Accession revealed as highly resistant by test. L136 is a red-seeded prolific vine type plant. Check is Fordhook Bush.

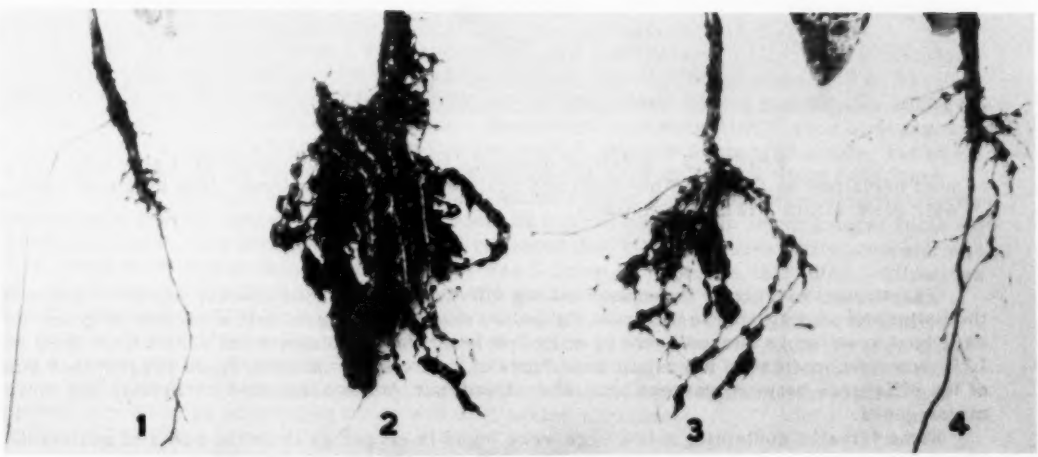

FIGURE 2. Segregation as seen in the field. Plants 1, 3 and 4 are H110-10 (F_3). Plant 1, class 1 resistant. Plant 2, Fordhook Bush (check) class 5 susceptible. Plant 3, class 5 susceptible. Plant 4, class 2 resistant.

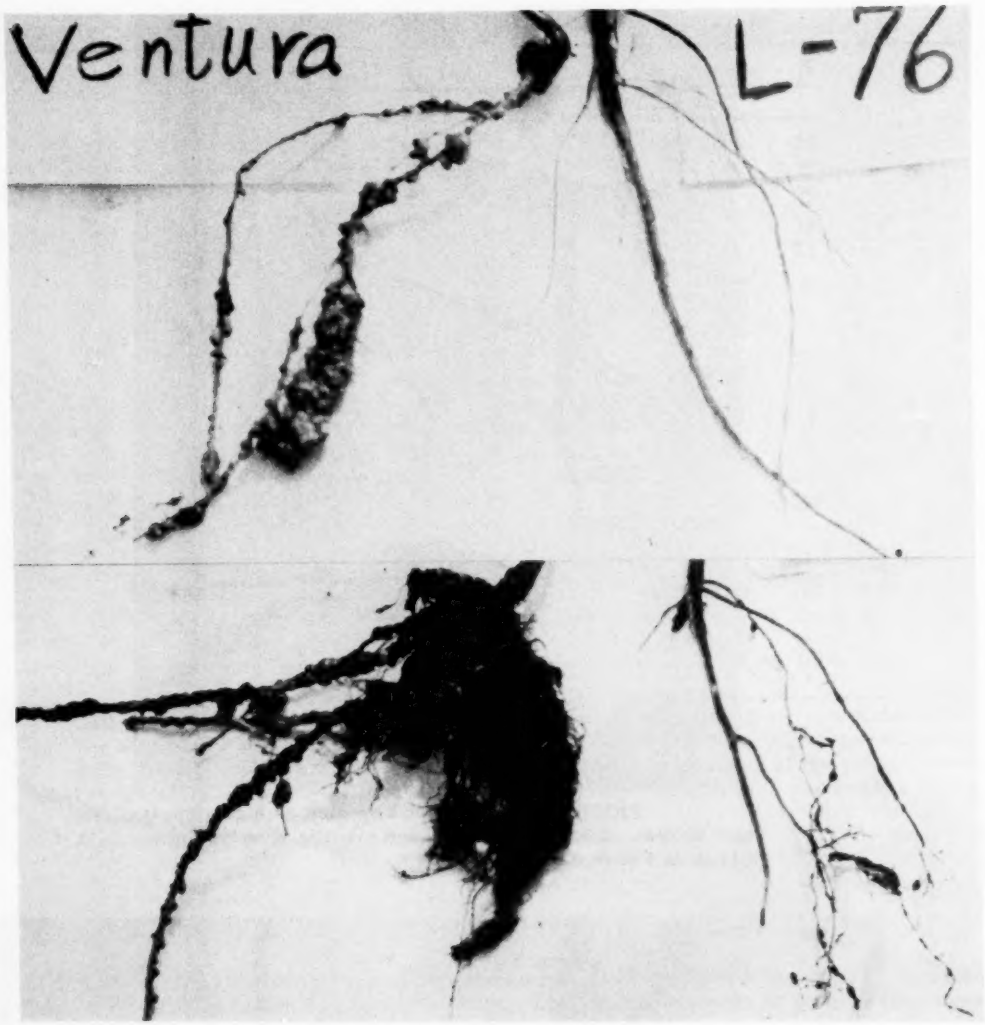

FIGURE 3. Effect of moderate (upper) and severe (lower) attack by root-knot nematode on a susceptible (left) and resistant (right) lima bean. The plants of each pair grew within a two-foot distance.

Variations in levels of resistance among different homozygous stocks, and differences in the pattern of segregation in different F_3 and F_4 families, suggest that a number of genes influence host reaction to the root-knot species involved. In hybrids between susceptible lines and L76, however, pattern of segregation and rate of recovery of parental types suggest that most of the difference between susceptible and resistant parents is accounted for by only one or two major genes.

Some females containing a few eggs were found in larger galls on the roots of resistant plants. Nematode larvae apparently penetrate the roots of plants with "practical" resistance but growth and reproduction appear to be limited in proportion to the level of resistance.

HAWAII AGRICULTURAL EXPERIMENT STATION, AND UNIVERSITY OF CALIFORNIA

SOIL FUMIGATION FOR CONTROLLING ROOT-KNOT NEMATODES ON TOMATOES FOR TRANSPLANT AND FOR FRESH FRUIT PRODUCTION[1]

J. M. Good and A. E. Steele[2]

Summary

Broadcast applications of DD mixture at 15 and 20 gallons per acre, 83 percent EDB at 4.4 and 5.9 gallons per acre, and DBCP (30 percent by weight) on clay granules at 346 and 692 pounds per acre, on land moderately to heavily infested with root-knot nematodes (Meloidogyne incognita) did not give the required degree of root knot control for certification of tomato plants grown for transplanting as root-knot free. However, chemical treatments gave economic control of root-knot nematodes and significant yield increases for tomato fruit. The higher rates of DD mixture and 83 percent EDB gave better root knot control and larger yields than lower rates. The lower rate of granular DBCP was as effective as the higher rates of DD mixture and 83 percent EDB. Only at the higher rate of DBCP was there an indication of phytotoxicity.

Root-knot nematodes of several species are a limiting factor in the production of tomatoes and tomato transplants in South Georgia. Until recently crop rotation and the use of newly cleared land have been the most practical means for their control.

However, crop rotations have not been entirely satisfactory because few crop plants are resistant to root-knot nematodes, and suitable newly cleared land is becoming scarce. This experiment was initiated to evaluate the possible uses of soil fumigants as an alternate method for controlling root-knot nematodes in tomato plant fields and in fields of tomatoes grown for market.

The requirements for control of root knot in transplant fields is much more exacting than for production of fruit because transplants must be completely free of root knot before they can be certified as disease-free in Georgia, whereas only approximately 85 percent control is generally considered adequate for fruit production. An easily applied granular material capable of controlling root-knot nematodes from the soil surface to a depth of about 1 foot would be desirable for producing tomato transplants; therefore, in this experiment granular DBCP was compared with DD mixture and EDB for this purpose.

In a series of field tests over a 3-year period, Lear and Thomason (2) found that fumigation of California soils with DD mixture (dichloropropene and dichloropropane), EDB (ethylene dibromide), and DBCP (1,2-dibromo-3-chloropropane) increased tomato yields by as much as 19.5 tons, though results were quite variable. On several occasions DBCP used at dosages of 1.5 gallon per acre broadcast or 0.6 gallon per acre in the row depressed yields, but at rates of 0.75 gallon per acre broadcast and 0.3 gallon per acre in the row yield reductions were not evident. Even at these low rates these authors found some indication of phytotoxicity, evidenced by a coarse, brown texture of the roots of injured plants with fewer lateral roots than healthy plants. McBeth and Bergeson (3) reported that 1,2-dibromo-3-chloropropane was 8 to 16 times more active than DD mixture and 2 to 5 times more active than EDB. Gilpatrick et al. (1) reported that DBCP could be effectively used at shallower placement depths than either DD mixture or EDB because of its lower volatility. Granular formulations of DBCP were equally as effective as liquid formulations at depths of 4 to 8 inches but were less effective at a 2-inch depth. Other reports (4, 5, 6, 7) indicated that solid carriers for both DD mixture and EDB give nematode control comparable with that from liquid nematocides, and suggested a number of advantages in the use of granular carriers.

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[1]Cooperative investigation between the United States Department of Agriculture, Agricultural Research Service, and the Georgia Agricultural Experiment Stations.

[2]Nematologist and Assistant Nematologist, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Tifton, Georgia.

MATERIALS AND METHODS

The soil of the experimental field, a Tifton sandy loam, was moderately to heavily infested with Meloidogyne incognita (Kofoid & White) Chitwood, 1949. All the fumigants were applied 2 weeks before planting. Liquid nematocides were injected with pressurized tractor-drawn equipment with injection chisels spaced 12 inches apart; nematocide-impregnated granules were spread on the soil surface with a fertilizer spreader and cut in with a disc harrow. A drag followed both the injection rig and the disc harrow to insure an adequate soil seal. Control plots were disc-harrowed only, so that all plots were treated alike as far as possible. The nematocides and application rates of each chemical were 1) 83 percent EDB (83 percent by weight of ethylene dibromide) at 4.4 and 5.9 gallons per acre; 2) DD mixture (50 percent by weight of 1,3-dichloropropane and about 50 percent by weight of 1,2-dichloropropene) at 15 and 20 gallons per acre; and 3) 10 percent by weight of DBCP (97 percent by weight of 1,2-dibromo-3-chloropropane) on clay granules at 346 and 692 pounds of granules per acre, respectively equivalent to 2 and 4 gallons of technical Nemagon per acre.

There were four randomized replications of each nematocide rate and eight replications of unfumigated control plots; the plots were 100 feet long by 4 1/2 feet wide. Three 18-inch rows of Stokescross No. 5 tomato seed were planted in each plot on March 25, 1957 with field seeding equipment[3]. Seeds were spaced approximately 1 inch apart in the drill.

Seven weeks after planting, stand counts were made on the number of tomato plants in every third foot along the middle row of each plot. Afterwards, approximately 100 plants were dug from each plot and scored for incidence of root knot, using an index system of 0 to 3 (0, clean; 1, light; 2, moderate; 3, heavy). The plants were then weighed to obtain the mean weight of plants for each plot.

The plots were thinned on May 23, 1957 by removing the middle row and plants in excess of one plant every 3 feet in the two outside rows of each plot. The remaining plants were side-dressed with a complete fertilizer and allowed to mature. Yield data were taken for the number and weight of mature fruit harvested; following the last picking the plants were dug and the roots scored for incidence of root knot, using an index system of 0 to 4 (0, clean; 1, light; 2, moderate; 3, heavy; 4, severe).

RESULTS

Root knot control in the tomato transplant production phase of the experiment was better in all fumigated plots than in the controls, and root knot indices were lower for the higher application rates of each chemical (Table 1). The lower rate of granular DBCP appeared about as effective as the higher application rates of DD mixture and 83 percent EDB. In the plots receiving the 692-pound application of granular DBCP, 93 percent of the plants were free from root knot; however, even this degree of control would not meet certification standards of the Georgia Department of Entomology. Though not significant, plant stands in fumigated plots tended to be better than in unfumigated plots. In all cases plants grown in fumigated soil had a higher mean plant weight than control plants, but this increase was significant for only the 692-pound rate of granular DBCP. Plants from fumigated plots were also more uniform in size than those from control plots (Fig. 1).

On plants grown for fruit production (Table 2), the root knot index of plants from plots treated with the highest rate of DBCP was significantly lower than that of any other treatment at the end of the season. Both the 346- and 692-pound granular DBCP applications gave significant yield increases, but the lower rate was apparently as effective as the 692-pound rate. Furthermore, the 346-pound application gave yield increases and root knot control that compared favorably with the higher rates of 83 percent EDB and DD mixture. DD mixture and granular DBCP at 20 gallons and 346 pounds per acre, respectively, more than doubled fruit yield, while 83 percent EDB at 5.9 gallons per acre tripled yield (Table 2).

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[3]Appreciation is expressed to Dr. H. I. Borders, Plant Pathologist, Agricultural Research Service, United States Department of Agriculture, who furnished field seeding equipment comparable with that used by commercial plant growers.

Table 1. Effect of soil fumigants on tomato plant stands, growth, and root knot control.

Soil treatment	Application rate (per acre)	Plant stand (mean number per foot)	Plant growth (mean weight per plant - ounces)	Percent of plants free of root knot	Mean root knot index (0 to 3)
DD mixture	15 gal.	8.2	0.78	62	0.61
	20 gal.	5.3	0.73	75	0.26
83 percent EDB	4.4 gal.	4.9	0.65	48	0.57
	5.9 gal.	6.2	0.79	85	0.17
Granular DBCP[a]	346 lb.	7.3	0.70	78	0.25
	692 lb.	6.3	0.94	93	0.07
Unfumigated control	--	4.6	0.54	2	1.8
Sig.		n.s.	10%	-	-
LSD .01		-	-	39	0.51
LSD .05		-	0.32	29	0.37
LSD .10		-	0.27	-	-

[a] 10 percent by weight technical material.

Table 2. Effect of soil fumigants on number and weight of tomatoes and on root knot control on mature tomato plants.

Soil treatment	Application rate (per acre)	Mean number of tomatoes harvested per plot	Tomato yields (pounds per acre)	Mean root knot index (0 to 4)
DD mixture	15 gal.	305	5,794	3.79
	20 gal.	476	7,863	3.70
83 percent EDB	4.4 gal.	376	6,816	3.59
	5.9 gal.	553	10,350	2.14
Granular DBCP[a]	346 lb.	525	8,966	2.11
	692 lb.	508	8,848	0.55
Unfumigated control	---	170	3,040	3.96
LSD .01		339	5,421	1.28
LSD .05		251	4,007	0.95

[a] 10 percent by weight technical material.

FIGURE 1. Root knot control in tomato plant field. Left, plot treated with 692 pounds per acre granular DBCP cut in with disc harrow; right, unfumigated control plot.

Pronounced phytotoxicity was not encountered in this experiment. There was a slight indication of toxicity from DBCP at the higher rate, but this appeared only on mature plants at harvest, not on the transplants. The roots of the injured plants were coarse, dark brown, with fewer lateral roots than is normal for healthy plants. This condition was not evident on plants treated with granular DBCP at 346 pounds per acre. Yield data (Table 2) also indicate that the higher rate of DBCP may have caused some stunting. Root knot control in these plots was very good, but tomato yields were generally lower than from plots treated with 83 percent EDB.

DISCUSSION

The data from this experiment indicate that soil fumigation with such chemicals as DD mixture, EDB, and DBCP will not give absolute control of root knot nematodes on moderately to lightly infested land, though it will be fairly easy to produce plants more than 75 percent free with the remaining plants only lightly infected. If the soil is less heavily infested, a greater percentage of nematode-free plants can be produced, but except for very light infestations, it is doubtful that soil fumigation alone will reduce nematode populations sufficiently to produce 100 percent root-knot-free transplants, which is required by many regulatory organizations. This suggests the use of root-knot nematode-reducing rotations prior to soil fumigation and growing tomato transplants. Such a system is being used successfully by several commercial growers in south Georgia. The practical value of fumigating newly cleared land before growing tomato plants has not been explored, but it sometimes happens that such land has a light to moderate infestation of root-knot nematodes, particularly if it has been cultivated and then allowed to revert to native vegetation. In such cases, soil fumigation might facilitate the growing of acceptable transplants where this could not be done otherwise.

In this experiment the granular formulation of DBCP, which was incorporated into the soil with a disc harrow, gave good control of root-knot nematodes. However, this method of applying granular nematocides is probably not the most efficient because of the difficulty of mixing the granules deeply enough into the soil, particularly in heavier soils. Gilpatrick et al. (1) found that granular formulations of DBCP were less effective when applied at 2-inch depths than when applied at 4 inches or more. The disc harrow incorporation method undoubtedly leaves a certain proportion of the granules in the upper 2 inches of soil where the nematocide volatilizes before producing an appreciable nematocidal effect. Because of its lower volatility, however, escape of DBCP would not be so great as for EDB and DD mixture similarly applied.

In addition to the possible uses of nematocides in the production of tomato transplants, the data indicate that substantial yield increases of tomatoes can be obtained from commercial applications of soil fumigants even where root knot control is far from perfect at the end of the season. It should be noted, however, that root-knot nematode control on these plots earlier in the season (as shown by the root-knot index figures of Table 1) was very good.

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FUMIGATION WHEN TRANSPLANTING NURSERY STOCK

P. M. Miller

Addition of a nematocide at time of transplanting will control the nematodes present at the planting site and prevent spread of nematodes from infested roots long enough to permit establishment of a satisfactory primary root system. Tests were started to determine phytoxicity of this type of treatment.

The nematocide used, 1,2-dibromo-3-chloropropane -- which is sold as Nemagon or Fumazone and is referred to hereafter as DCP -- was selected because its effective state is gaseous and it thus needs no water to distribute it through the soil. Where water is needed in the transplanting, V-C 13 (O-2,4-dichlorophenyl-O,O-diethyl phosphorothioate) or DCP could be used.

An oil can ejecting a given volume each time is a convenient and quick applicator for treating a large number of plants. Each ejection by the type used released 2.2 ml of a mixture composed of 9 parts water and 1 part of a 50 percent emulsifiable concentrate of DCP. Each ejection contains 0.11 ml actual DCP, which is considered an adequate dosage for 1 cubic foot of soil, or sufficient for one ejection to treat a planting hole 12 to 18 inches in diameter and 6 to 9 inches deep. The emulsifiable concentrate could be diluted differently for use in different dispensers, depending upon the volume of ejection.

The fumigant was added at time of planting for some plants and shortly after planting for other plants. If added at planting time, one-half to three-quarters of the soil was first placed around the roots. Then the nematocide was distributed on top of this soil, using one ejection of 2.2 ml from the oil can for each estimated cubic foot of the planting hole, or at approximately 1-foot intervals around the plant. Then the remainder of the soil was added. This is the preferred method of application. Where the roots were covered before treatment could be made, one hole 6 inches deep was made in each square foot of the surface of the planting hole and one ejection placed at the bottom of each hole. Then the treatment holes were filled with soil and tamped to seal the surface and prevent escape of the fumigant.

DCP was added at the time of planting or soon after planting of apple (Pyrus malus), peach (Prunus persica), pear (Pyrus communis), plum (Prunus domestica), Japanese yew (Taxus cuspidata), white oak (Quercus alba), northern red oak (Quercus borealis), white dogwood (Cornus florida), white pine (Pinus strobus), red maple (Acer rubrum), and arrowwood bush (Viburnum dentatum), in 1957 or 1958.

RESULTS: There was no evidence of phytotoxicity of DCP added at the time of planting. No response attributable to nematode control was noted, but no treatments were made in an area known to suffer serious nematode injury.

DISCUSSION: The most important injury from nematodes occurs just after planting time. Application of a nematocide at planting time provides an economical and convenient method for landscape gardeners, retail nurserymen, and homeowners to combat nematode injury to nursery stock without any change in planting techniques. The method was found, in this test, to be safe for use around roots of widely divergent types of plants. However, as in any new practice, addition of either DCP or V-C 13 to the planting hole should be tried first on a small scale, particularly with the lesser-known types of plants. These fumigants have been reported as safe for application in the soil around established plants of many types of nursery stock. However, it is obviously impossible, in one area or one experiment, to test their safety for all of the hundreds of types and varieties of plants sold by nurseries.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CONNECTICUT

CERTAIN FATTY ACIDS AND HEXADECYLAMINE AS NEMATOCIDES[1]

C. A. Loos[2]

Summary

Fatty acids (heptanoic-undecylenic) are nematocidal within the range of 25 to 1000 ppm when applied in aqueous emulsion to surfaces, as judged by laboratory contact tests. Nemorad III (pelargonic acid formulation), hexadecylamine (technical) and hexadecylamine acetate, applied as a surface drench to soil at extremely high application rates, were ineffective in controlling the root-knot nematode. In contrast, Nemagon, a fumigant applied by injection at 1 1/4 gallons per acre, gave a very high degree of control. Nemorad III and hexadecylamine when incorporated into the soil by mixing gave a good degree of control of root-knot at application rates of 800 pounds per acre. Nemagon fumigant was ineffective when applied around root-knot infested tomato plants. A very high degree of control was achieved when Nemagon-treated soil was planted 9 days after treatment. Planting 2 days after fumigation required higher application rates for effective control than planting after a 10-day interval. Solutions of Nemorad III and hexadecylamine lose their nematocidal value when passed through 2 inches of garden loam. Yet the leachates, after passing through 6 inches of sand, are nematotoxic. The toxicants are apparently adsorbed on the organic matter and/or clay particles. The adsorption and surface activity properties of fatty acids and hexadecylamine make them useful as surface disinfestants or in combination with soil fumigants. Hexadecylamine (technical) and hexadecylamine acetate were about 6 and 25 times more toxic than Nemorad III on contact with Panagrellus redivivus.

Certain fatty acids in the intermediate-numbered carbon groups were found to possess high nematocidal activity by Tarjan and Cheo (2). Heptanoic (enanthic), caprylic (octanoic), pelargonic (nonanoic), capric (decanoic), and undecanoic acids at 1000 ppm aqueous concentrations were toxic, within a few minutes, to the nematode Panagrellus redivivus in in vitro tests. Dipping cyst-infested (Heterodera tabacum) burlap in 2 percent concentrations of these emulsified acids caused total inactivation of larvae in the cysts. Nematocidal activity appears maximum when an intermediate number of carbons is present as an aliphatic straight chain. Tarjan and Cheo (2) also obtained excellent nematocidal results in in vitro tests, using P. redivivus as the test organism, with certain salts of fatty acids. Aqueous preparations containing 1000 ppm of the ammonium salts of heptanoic, caprylic, pelargonic, capric and undecylenic acids inactivated the test nematode within 2 hours, while zinc, sodium, and methy undecylenate were in the lower range of nematotoxicity. Butyl, copper, and potassium undecylenate were non-toxic under similar conditions.

The writer made additional tests with 11 metallic pelargonates of aluminum, ammonium, barium, calcium, lithium, magnesium, nickel, potassium, sodium, strontium, and zinc, obtained from the Martheco Chemical Company, New York. Emulsifiable concentrates of the salts were made as follows:

Pelargonate	-- 1 part
Tween 40	-- 1 part
Isopropyl alcohol	-- 0.865 parts

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The concentrate was diluted to make aqueous emulsions containing 1000 and 100 ppm of pelargonate, in Beltsville-type Syracuse dishes. Ten individuals of P. redivivus were transferred to each dish. The preparations were examined at intervals of 1/2, 1 1/2, 3 and 24 hours. At the 1000 ppm concentration of ammonium pelargonate the nematodes were totally inactivated in less than 1/2 hour, but at 100 ppm there was no nematotoxicity. In emulsions of the other ten compounds the nematodes continued to remain active up to 24 hours, when observations ceased.

The degree of toxicity of the water soluble salts of the fatty acids to the test nematode, P. redivivus, is set out in Table 1. Of special note is the high toxicity of the ammonium salts and the low to total absence of toxicity of the other test compounds. A probable explanation is that the ammonium salts dissociate rapidly in aqueous solution to form the free fatty acid which, being insoluble and lighter than water, floats as oily globules on the surface of the solution. It is probable that the nematotoxic agents are the highly toxic free heptanoic, pelargonic, caprylic, capric and undecylenic acids to which the ammonium salts of these acids dissociate, and not the ammonium salts themselves. Potassium, sodium, and lithium salts do not appear to dissociate similarly and are therefore non-nematotoxic.

Tarjan and Cheo (2) obtained highly satisfactory kills of larvae in cysts of Heterodera tabacum with pelargonic acid, undecanoic acid, caprylic acid, capric acid, undecylenic acid, ammonium pelargonate and ammonium undecylenate, while potassium and sodium undecylenate proved non-toxic. It appears reasonable to attribute the success achieved with the ammonium salts to toxicity following dissocation of the compound and not the compound itself.

A more stable, therefore less nematocidal preparation of ammonium pelargonate was formulated by the Mallinckrodt Chemical Works as Nemorad 1, with the following composition:

Pelargonic acid	-- 5 parts
Ammonium hydroxide (28%, NH_3)	-- 1 part
Tween 40	-- 1 part
Sorbitol	-- 1 part
Water	-- 9.6 parts

This formulation contains 27 percent pelargonic acid as the ammonium salt.

Nemorad 1 showed lower nematocidal value in contact tests with Panagrellus redivivus, requiring 4000 ppm of the formulation to totally inactivate the test organism in 24 hours as compared with 750 ppm of a preparation containing emulsified pelargonic acid.

Pelargonic Acid as a Soil Disinfestant

For these experiments, the chemical was obtained as an emulsifiable concentrate formulated as the product Nemorad III by the Mallinckrodt Chemical Works. It contains 35 percent pelargonic acid and makes a very stable aqueous emulsion. The experiment was divided into five series. Meloidogyne incognita-infested soils free from roots were used in all series.

In experiments 1 to 3 the infested soil was placed in No. 10 cans (6 inches in diameter by 6 inches in depth). In addition to Nemorad III (pelargonic acid) comparative treatments were made with Nemagon (Shell Chemical Co.) liquid and a combination of Nemorad III and Nemagon. Nemorad III was applied as a surface drench of 1000 ml aqueous preparations containing the requisite amount of pelargonic acid. Nemagon was applied in all cases at 15 gallons per acre of a mixture of the requisite amount of Nemagon in a diluent of kerosene oil. The fumigant was applied 4 inches below soil level at four points in each can.

Tomato (Lycopersicum esculentum L. hort. var. Valiant) was used as the test plant in estimating the degree of nematocidal activity. At examination time the plants were carefully washed clean of soil and the degree of root-galling estimated according to Tarjan and Cheo's (2) rating code which is as follows:

Rating Code for Galling of Roots.

Class 0 -- No visible galls
1 -- 1-25 percent of root tissue galled
2 -- 26-50 " " " " "
3 -- 51-75 " " " " "
4 -- 76-100 " " " " "

Evaluation of Degree of Control.

9 -- No galling -- excellent
7 -- Limited galling -- very good
5 -- Average root-knot index 2-3 points lower than average of check plants -- good
3 -- Average root-knot index one point below that of check plants -- fair to poor
1 -- Average root-knot index of treated plants same as that of checks -- ineffective

Experiment 1: Cans of infested soil were treated as shown in Table 2. Each treatment had five replications. On the tenth day following treatment the cans were planted with 5-week-old tomato seedlings which were topped back to a uniform height immediately after planting. Seven weeks later the plants were examined for phytotoxicity, growth, and root-galling.

There was no apparent phytotoxicity with either Nemagon up to 10 gallons per acre or Nemorad III at 4571 pounds per acre (pelargonic acid 1600 pounds per acre). The extra large size of the plants in the Nemagon-Nemorad treated series was a marked feature. The roots of this series had grown through the drainage holes in the bases of the cans and were feeding on nutrients in the soil below. None of the plants in the other treatments grew so vigorously.

A feature of this experiment was the absence of phytotoxicity of Nemorad III to the tomato plants, even at the high application level of 4571 pounds (1600 pounds pelargonic acid) per acre. However, this level gave no control of root-knot eelworm, the plants being affected as severely as the untreated checks. As regards Nemagon the excellent control achieved at the rate of 1 1/4 gallons per acre and the absence of phytotoxicity up to 10 gallons per acre were noteworthy. The same excellent control resulted from the Nemagon-Nemorad application, though the activity was obviously due to the Nemagon. It appears likely that the two substances together may have induced additional nutrient effects.

Experiment 2: This experiment was similar to experiment 1 (Table 3) except that tomato seedlings were planted a day after soil treatment. The shorter interval between treatment and planting induced phytotoxicity at the 1600 pounds per acre pelargonic acid application level. For total control of root knot an application rate of 5 gallons of Nemagon per acre was required, in comparison with 2 1/2 gallons when fumigation was done 10 days before planting. No injury resulted from applications of Nemagon and the plants were significantly larger than those in the other treatments.

Experiment 3: In this experiment the tomato plants were established in the infested soil 2 weeks before treatment with nematocides (Table 4). The treatments were similar to those of experiments 1 and 2, but at the time of treatment the root systems of the plants were heavily infested and the root-knot nematode females were approaching maturity. Treatment was, therefore, for therapeutic evaluation.

Nemagon up to 10 gallons per acre did not cause injury to the tomato seedlings even though applied around the roots of the growing plant. Nemorad III under similar conditions proved phytotoxic at the 400 pound per acre application level. In the series where Nemagon 2 1/2 gallons/Nemorad III 200 pounds per acre were applied there was no injury to the tomato plants, whereas Nemagon 2 1/2 gallons/Nemorad III at 400 pounds per acre caused the death of four of the five replicates. The phytotoxic agent was undoubtedly Nemorad III.

The failure to control root knot or effectively reduce infection of plants already infected prior to fumigation with Nemagon demonstrated the need of fumigation of infested soil before the plants are established. Obviously, Nemagon, like most fumigants, has limited residual value and is effective for a short period only. Nematodes established in root tissue are unaffected and continue their life cycle unchecked to reinfest the soil. Although Nemogon is an efficient soil nematocide, it has no value as a therapeutant and little value as a single application to heavily root-knot infested tomato plants. There was no apparent nutrient effect following treatment.

Experiment 4: Infested soil was placed in No. 10 cans and Nemorad III preparations added to the soil as 300 ml aqueous formulations containing the requisite concentrations of the chemical and well mixed into the soil. Three replicates were made per treatment. Treatments were similar to those used in experiments 1 to 3 except that, in this experiment, (see Table 5), Nemagon was omitted. Tomato plants (5 weeks old) were planted 48 hours after treatment. Reduction of galling was apparent at the 200 pounds per acre rate of application. Fairly good control was established with 400 pounds application, excellent control with 800 pounds, and

Table 1. Degree of nematotoxicity of the water-soluble salts of some fatty acids to the test nematode Panagrellus redivivus in "in vitro" tests. Tests were made with concentrations of 1000 ppm of the salt.

Fatty acid salt	Nematotoxicity
Ammonium pelargonate	Very high
Ammonium heptanoate	Very high
Ammonium caprylate	Very high
Ammonium caproate	Very high
Ammonium undecylenate	Very high
Lithium pelargonate	None
Potassium pelargonate	None
Potassium undecylenate	Very slight
Sodium pelargonate	None
Sodium undecylenate	None

Table 2. Nematocidal effect of Nemorad III and/or Nemagon applied to Meloidogyne incognita-infested soil 9 days before planting with 5-week-old tomato plants. Evaluations made 7 weeks after planting. Application of Nemorad III expressed as pelargonic acid weight.

Treatment	Average shoot length (inches)	*Galling Class	*Galling Evaluation	Phytotoxicity
Nemagon ½ gal./Acre	8.8	2	5	None
Nemagon 1¼ gal./Acre	9.2	1	7	"
Nemagon 2½ gal./Acre	9.8	0	9	"
Nemagon 5 gal./Acre	9.6	0	9	"
Nemagon 10 gal./Acre	10.0	0	9	"
Water check	3.8	4	1	"
Emulsifier check	4.0	4	1	"
Kerosene 14½ gal./Acre	3.0	4	1	"
Nemorad III 25 lbs/Acre	5.2	4	1	"
" 50 lbs/Acre	5.4	4	1	"
" 100 lbs/Acre	5.4	4	1	"
" 200 lbs/Acre	4.4	4	1	"
" 400 lbs/Acre	5.8	4	1	"
" 800 lbs/Acre	4.8	4	1	"
" 1600 lbs/Acre	3.4	4	1	"
Nemorad III 200 lbs+Nemagon 2½ gal./Acre	25.4	0	9	"
" 400 lbs+Nemagon 2½ gal./Acre	18.6	0	9	"

*See rating codes in text.

Table 3. Nematocidal effect of Nemorad III and/or Nemagon applied to Meloidogyne incognita-infested soil one day before planting with 5-week-old tomato plants. Evaluations made 7 weeks after planting. Application of Nemorad III expressed as pelargonic acid weight.

Treatment	Average shoot length (inches)	*Galling Class	*Galling Evaluation	Phytotoxicity
Nemagon ½ gal./Acre	11.6	3-4	1	None
" 1¼ gal./Acre	11.0	3	3	"
" 2½ gal./Acre	10.4	0-1	5-9	"
" 5 gal./Acre	10.0	0	9	"
" 10 gal./Acre	11.8	0	9	"
Water check	8.0	4	1	"
Emulsifier check	8.0	4	1	"
Kerosene 14½ gal./Acre	8.0	4	1	"
Nemorad III 25 lbs to 800 lbs/Acre	9.2	4	1	"
" 1600 lbs./Acre	Plants died 2 days after planting.			
" 200 lbs+Nemagon 2½ gal./Acre	12.2	0-1	5-9	"
" 400 lbs+ " " " /Acre	11.4	0-1	5-9	"

*See rating codes in text.

Table 4. Nematocidal effect of Nemorad III and/or Nemagon applied to Meloidogyne incognita-infested soil in cans in which tomato plants had been established for 2 weeks. Evaluation made 5 weeks after treatment. Application of Nemorad III expressed as pelargonic acid weight.

Treatment	Plant height (inches)	*Galling Class	*Galling Evaluation	Phytotoxicity
Nemagon ½, 1¼, 2½, 5 or 10 gal./Acre	8.4-11.8	3-4	1-3	None
Water check	11	4	1	"
Emulsifier check	9 -10.8	4	1	"
Kerosene 16½ gal./Acre	8.8-10.6	4	1	"
Nemorad III 25, 50, 100 or 200 lbs/Acre	9.6-11.6	4	1	"
Nemorad III 600 lbs/Acre	10	4	1	3 dead
Nemorad III 800 or 1600 lbs/Acre				All dead
Nemorad III 200 lbs+Nemagon 2½ gal./Acre	9 -12	3-4	1-3	None
Nemorad III 400 lbs+Nemagon 2½ gal./Acre				All dead

*See rating codes in text.

Table 5. Nematotoxicity of Nemorad III when well mixed into infested soil in cans as 300 ml aqueous formulations and 5-week-old tomato plants planted 48 hours later. Plants evaluated for galling 6 weeks after planting. Application of Nemorad III expressed as pelargonic acid weight.

Treatment	Plant height (inches)	*Galling Class	*Galling Evaluation	Phytotoxicity
Water check	13-6	4	1	None
Emulsifier check	9-6	4	1	"
Nemorad III 25, 50 or 100 lbs/Acre	8.3-14	4	1	"
" III 200 lbs/Acre	17	2-3	3-5	"
" III 400 lbs/Acre	14-6	1-2	5	"
" III 800 lbs/Acre	18-6	0-1	5-9	"
" III 1600 lbs/Acre	19-6	0	9	"

*See rating codes in text.

Table 6. Nematocidal activity of the "oil" layer and the "clear" liquid found after the addition of hexadecylamine (techn.) to hot water. The "clear" liquid was diluted with water for testing, while the "oil" layer was used as an aqueous emulsion. Ten Panagrellus redivivus worms were added to dilutions contained in Beltsville-type Syracuse dishes.

	Percentage worms inactived after ½ hour	1½ hours	3 hours	24 hours
"Clear" solution				
Water check	0	0	0	0
10,000 ppm (1%)	100	-	-	-
1,000 " (0.1%)	30	100	-	-
500 " (0.05%)	20	20	30	30
250 " (0.025%)	0	0	0	20
120 " (0.0125%)	0	0	0	0
"Oil" layer (emulsified)				
Emulsifier only	0	0	0	0
10,000 ppm (1.0%)	100	0	-	-
1,000 " (0.1%)	100	-	-	-
500 " (0.05%)	100	-	-	-
250 " (0.025%)	100	-	-	-
125 " (0.0125%)	100	-	-	-
62 " (0.0062%)	80	100	-	-
12 " (0.0012%)	0	0	10	40

Table 7. Comparative nematotoxic action of hexadecylamine emulsion (1 part hexadecylamine techn., 1 part Tween 40, and 1 part isopropyl alcohol w/w) and hexadecylamine acetate solution against Panagrellus redivivus.

Dilutions	Percentage inactivation of P. redivivus after (hours) Hexadecylamine ½ Tech.	Hexadecylamine 1½ Tech.	Hexadecylamine 3 Tech.	Hexadecylamine 24 Tech.	Hexadecylamine ½ Acetate	Hexadecylamine 1½ Acetate	Hexadecylamine 3 Acetate	Hexadecylamine 24 Acetate
1000 ppm	100	-	-	-	100	-	-	-
500 "	100	-	-	-	100	-	-	-
250 "	100	-	-	-	100	-	-	-
100 "	100	-	-	-	100	-	-	-
50 "	0	20	100	-	100	-	-	-
25 "	0	20	100	-	60	100	-	-
10 "	0	0	10	100	60	100	-	-
5 "	0	0	0	0	0	100	-	-
2½ "	0	0	0	0	0	100	0	-
1 "	0	0	0	0	0	0	0	30
Water check	0	0	0	0	0	0	0	0
Emulsion check	0	0	0	0	0	0	0	0

total control with 1600 pounds per acre.

These results show that for control of root knot to a 6-inch depth over 2285 pounds of Nemorad III (800 pounds of pelargonic acid) well incorporated into the soil are necessary. Applications as surface drenches are of no value for subsurface kill.

Hexadecylamine and its Related Compound Hexadecylamine Acetate

Technical hexadecylamine is not soluble in water at room temperature, but when added to hot water (70° C) it melts to an oily layer which floats on the surface of the water. In vitro tests with Panagrellus redivivus showed that the oil layer was the nematocidal agent. Hexadecylamine (technical) at 1 gm to 100 ml of hot water was added to a separation funnel and allowed to remain, after stirring, for 1 hour at 80° C in an incubator. The clear liquid and the oily layer were then drawn off separately and the latter was made up with water to the volume of the clear liquid, after the addition of emulsifying agents. Both emulsion and clear liquid were assumed to contain 1 percent of the nematocidal agent and dilutions were made on this assumption. Table 6 shows the results of in vitro tests of the two preparations with P. redivivus as the test organism. A dilution containing 62 ppm of the oil caused total inactivation of the test nematode after 1 1/2 hours immersion, whereas 1000 ppm of the clear liquid were required for comparable results. The oil layer obviously contained the nematocidal agent and apparently there is a slight solubility in water judging from the inactivation of the nematode in the 1000 ppm concentration.

Hexadecylamine acetate, as prepared by Mallinckrodt Chemical Works, dissolves very readily in hot water, but a 1 percent solution gelatinizes on cooling to room temperature. Heavier concentrations set to a very stiff gel when cooled to room temperature, but with no apparent separation of oil globules. Hexadecylamine acetate is 10 times more nematotoxic than technical hexadecylamine, though the latter, by itself, is of high toxicity. The acetate shows light nematotoxicity at the low concentration of 1 ppm. Table 7 compares the two compounds as contact nematocides for P. redivivus.

Nematocidal Value of Hexadecylamine in Soil

Neither hexadecylamine nor its acetate have any subsurface nematocidal value when applied as a surface drench to root-knot infested soil in cans. In a replicated experiment in which 1400, 700, 350, 175, 87, 44, 22, 11 and 5 pounds of hexadecylamine were applied as a surface application to root-knot infested soil in no. 10 cans, there was no reduction of root knot on tomatoes planted 2 days after soil treatment and examined 6 weeks later. Galling was as severe on the plants growing in cans treated at the 1400 pound per acre level as on the untreated plants. Slight phytotoxicity was observed at the 1400 pound rate.

Incorporation of technical hexadecylamine (in emulsion form) into soil by thorough mixing of 200 ml of an aqueous emulsion containing the requisite amount of chemical into no. 10 cans of root-knot infested soil gave slight control at 400 pounds and good control (25 percent of galled tissue as compared with 75 to 100 percent on untreated checks) at 800 pounds per acre. This degree of control was inferior, however, to that achieved with Nemorad III (pelargonic acid), as 800 pounds of technical hexadecylamine were required to achieve control comparable with that from Nemorad III at 400 pounds per acre.

Hexadecylamine acetate was equally ineffective when applied as a surface drench to infested soil. Incorporated into the soil at 800 pounds per acre it gave fair control of root-knot eelworm.

Adsorption of the Nematocidal Agent

The failure of Nemorad III (pelargonic acid), hexadecylamine technical, and hexadecylamine acetate to control root knot when applied as a soil drench, even at very high application rates, suggested that the active nematocidal agent was inactivated or adsorbed in the upper portion of the soil. This view was strengthened by the better control achieved when fairly heavy concentrations of the chemicals were mixed into the nematode-infested soil. However, it is possible that organic matter (acids, polysaccharides, etc.) reacted with the nematocides.

The adsorption properties of soil on hexadecylamine acetate in aqueous solution was demonstrated in the following experiment. A 1 3/4-inch diameter glass tube was fitted at one end with a 60-mesh screen and dry soil was added into the tube until the soil column reached 2 1/2 inches.

The tube was then lightly tapped until the soil packed down to 2 inches. A 4000 ppm (0.4 percent) aqueous solution of hexadecylamine acetate was added to the tube and the liquid which passed through was collected for in vitro tests with Panagrellus redivivus. The first collection of 70 ml was made after addition of 100 ml of the 4000 ppm solution, and six further collections of 50 ml were made, after addition each time of 50 ml. The final collection was made after an equivalent application of approximately 8000 pounds of hexadecylamine acetate per acre. None of the filtrates which passed through the 2-inch layer of soil had nematocidal effect on P. redivivus. After 24 hours' immersion in the filtrates, the nematodes were as active as those in the water check. For comparative check purposes a 0.004 percent (40 ppm) solution was made from the test chemical. In this preparation P. redivivus died in less than 1/2 hour.

A further experiment, also with hexadecylamine acetate, compared the adsorption properties of soil and sand. In this experiment 0.8 grams of hexadecylamine acetate were dissolved in 50 ml of water and the solution added to 6-inch columns of soil or sand set up in 1 3/4-inch diameter tubes. This amount of liquid wet the top 3 1/2 inches of the soil column. More water was added after 5 minutes, and the first 20 ml which passed through the column was collected for contact tests with P. redivivus. The application rate was approximately 4000 pounds per acre. While passage through soil caused inactivation of the nematocidal agent, the chemical retained its toxicity on passage through sand.

Surface Layer Disinfestation

The most effective nematocides, up to the present time, are fumigants, although one of the disadvantages of fumigants, especially when used against cyst nematodes (Heterodera spp.), is in the unsatisfactory kill obtained in the surface layer of soil. The ventilation of this layer prevents the retention of an adequate concentration of the toxic vapor for a sufficiently long time to give a satisfactory kill. Rolling of the soil immediately after application is of doubtful efficiency and light watering of the soil surface following injection of the fumigant is not often feasible. Peters (1) showed that there is a considerable loss of DD vapor from the surface layer of soil and that this loss is only moderately reduced by using a water seal. He suggested the combined application of a fumigant and a solubilized nematocidal chemical to deal with the cyst survivors in the upper 2 inches of soil.

A surface layer disinfestation trial with Nemorad III was set up under conditions rather similar to those of Peter's experiment. Root-knot infested soil (Meloidogyne incognita) was set up in a wooden box, 6 inches x 4 1/2 inches x 8 inches, which was made up of four rectangular superimposed wooden frames with sides 6 inches x 4 1/2 inches and 2 inches deep. The sections had side walls only, except for the bottommost section to which was fitted an open-mesh screen to hold the soil in the box and to allow for drainage. The four inner side walls of the box were lined with aluminum foil. A 500 ml aqueous preparation of Nemorad III, in sufficient concentration to give an application rate of 500 pounds per acre, was poured on the surface of the soil. The drenched soil, from which water drained through the lowest tray, was allowed to stand undisturbed for 24 hours during which time its height shrunk by 2 inches. Each of the three resulting 2-inch horizontal layers of soil in the superimposed box sections was divided into four equal portions. Each portion was added around the roots of a nematode-free tomato seedling which was carefully transferred from a small pot to a no. 10 can at inoculation time. Three weeks later the soil was washed out and the roots examined for root-knot infestation. The four plants to which soil from the uppermost 2-inch layer was added were completely free from nematode nodules. Nodulation on plants in the soil from the 2- to 4-inch and the 4- to 6-inch layers was as severe as that developing in untreated infested soil.

DISCUSSION

The fatty acids, though of high nematocidal activity in aqueous emulsion, lose their effectiveness by passage through soil. They can be of considerable contact value in the soil surface layer where fumigants alone are of limited effectiveness.

The high application rate of emulsified chemical needed for control of nematodes in soil and the requirement for thorough mixing for even distribution through the soil will make the large scale use of fatty acids as nematocides unlikely. They may, however, have exceptional value as disinfestants of farm machinery, containers, soil surfaces, etc.

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A COMPARISON OF TWO CONTACT NEMATOCIDE TEST METHODS[1]

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Summary

Two contact test methods of screening chemicals for nematocidal activity, using Panagrellus redivivus (Linn., 1767) Goodey 1945 as the test organism, were studied. A method utilizing an aeration-agitation apparatus is described and is compared with the contact test method developed by A. C. Tarjan and P. C. Cheo during 1952 to 1955, at the Rhode Island Agricultural Experiment Station. Comparative tests were made of both methods, using ten chemicals at a dilution of 1000 ppm of active ingredient. The nematocidal value of the chemicals was similar, though not identical, when evaluated by the two methods. The merits and disadvantages of each method are discussed from the standpoint of time and labor, testing of nematodes in various stages of the life cycle, and variability of results within and between both methods.

INTRODUCTION

During the past 10 years, substantial progress has been made in the discovery and formulation of soil fumigants and in their use in the field as nematocides. A few of the newer materials, e.g. 1,2-dibromo-3-chloropropane, may be used as post-planting nematocides with certain plants. Other chemicals of potential usefulness are those that could be used both as soil nematocides and equipment decontaminants, nematode repellents, or chemotherapeutants. In the continuing search for such new materials, various screening techniques are used. A preliminary rapid screening test, commonly called the contact nematocide test, is usually employed to eliminate large numbers of experimental chemicals prior to greenhouse and field testing. Tarjan and Cheo (7) used six different screening tests in their search for new types of anti-nematode chemicals, viz., contact, ovicide, foliar spray, soil amendment, stem injection, and repellency tests. It appeared advisable to continue studies on screening methods with the aim of increasing the probability that selected compounds would be effective in field use.

CONTACT NEMATOCIDE TESTS

A primary requirement for this test is a nematode of medium size, e.g. 1 mm or more in length, continually active, and readily cultured in large numbers in the laboratory. Peters (4) used Turbatrix aceti (Muller, 1783) v. aceti Peters, 1927 as his test organism. Gadd and Loos (3) cultured Panagrolaimus rigidus (Schneider, 1866) Thorne, 1937 readily in decaying organic matter. Cairns (1) cultured the mushroom-spawn nematode, Ditylenchus sp., a stylet type, in large numbers on mushroom spawn. Tarjan (6) suggested Panagrellus redivivus (Linn., 1767) Goodey, 1945, the sour paste nematode, cultured in an oatmeal medium as a favorable organism for contact tests. P. redivivus is now used by a number of workers in the United States for contact nematocide tests because of its adequate size, ease of culture, short life cycle, and continual activity.

METHODS

Contact Test A

In the contact test method of Tarjan and Cheo (7) chemicals were tested at concentrations of 1000 ppm (0.1 percent) and of 100 ppm (0.01 percent). Ten pre-adult or young adult nematodes were transferred individually to each test dish. Observations for total inactivation were

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made at seven time intervals over a 24-hour period. Total inactivation was verified by probing sensory structures with a wire needle and by lifting the nematode out of the test liquid for a few seconds. Chemicals were then rated according to the time required for total inactivation of all nematodes.

In this method, unless the suspension or emulsion, if the chemical is not water-soluble and has to be suspended or emulsified, is stable both the chemical and the nematodes sink to the bottom of the watch glass. Should that occur, the nematodes, instead of remaining in a predetermined 0.1 percent chemical suspension or emulsion, may be submerged in a sediment of undetermined concentration.

Contact Test B

A second contact test method was developed in 1956. For convenience this method, described below, is designated contact test B, and the method of Tarjan and Cheo (7) designated contact test A. Contact test B provides for continuous agitation and aeration of test liquids to avoid the settling out of suspended chemicals and nematodes, and avoids a possible oxygen deficit in the exposed test organisms. Santmyer (5) has shown a rather high oxygen requirement for Panagrellus redivivus. The method also utilizes all life stages of P. redivivus in evaluating test chemicals. It eliminates the long and tiring technique of transferring individual nematodes and avoids the possible injury through probing with a needle which is a requirement in contact test A. In contact test B, readings are taken after nematodes have been washed and transferred to water, thus allowing for the possibility of recovery from effects of the candidate chemical.

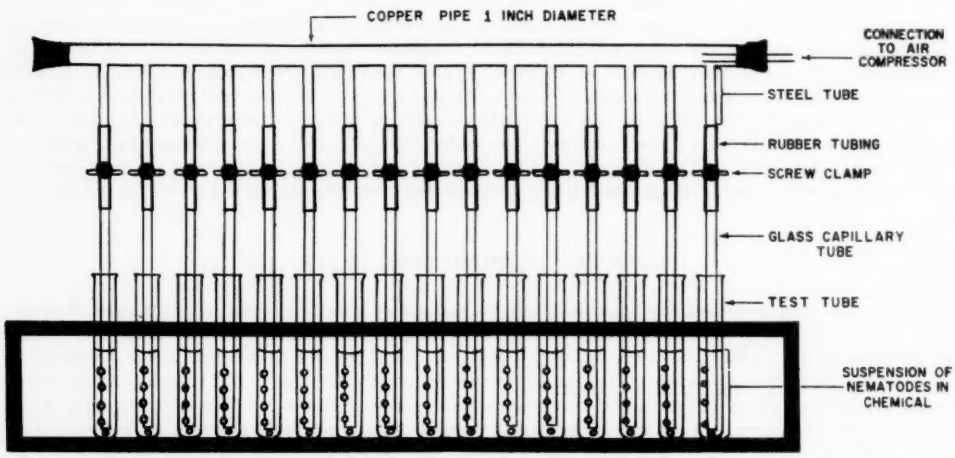

FIGURE 1. Diagram of aeration-agitation apparatus for use in contact nematocide tests.

Description of Contact Test B: Sixteen stainless steel tubes are fitted 2 inches apart along a vertical plane on a 1-inch diameter copper pipe (Fig. 1). Glass capillary tubes, 10 inches long, are attached to the steel tubes by means of short pieces of rubber tubing across which are also placed screw-type adjustable clamps. Each capillary tube is passed into a 24 by 200 mm test tube. Test tubes are held in a rack that can be raised or lowered. When in operation, the ends of capillary tubes just clear the bottom of the test tubes. Compressed air at 2 pounds per square inch is passed through water and then into the pipe supplying the 16 test tubes. Air emerging from the capillary tubes is regulated to a slow and uniform rate of bubbling by use of a screw clamp on the connector to each tube.

The chemical to be tested is prepared in solution, suspension, or emulsion at the desired concentration, and 40 ml added to each test tube half fills the tube. A suspension of nematodes from cultures grown in the manner described by Tarjan and Cheo (7) is prepared by adding two camel hair brush loads from the anastomosed mass of nematodes on the sides of the culture

dish to 25 ml water. One ml of this suspension, which contains various life stages of Panagrellus redivivus, is added to each tube of candidate chemical or control tube. Air flow is maintained throughout the test period. This air-bubbling keeps both nematodes and chemical particles in continuous movement. Excessive foaming is inhibited, where necessary, by adding 40 ppm of Dow Corning Antifoam A per tube. At this concentration Antifoam was found to be non-toxic to the nematodes.

For the total inactivation counts, made after 1/2, 1 1/2, 4, and 24 hours, 5 ml of test solution under agitation are withdrawn from the test tube and transferred to a large beaker containing 195 ml of water, and the mixture is swirled. This dilutes the 1000 ppm concentration to 25 ppm. The diluted suspension is passed through a medium porosity, fritted glass, Gooch crucible under vacuum after the method of Feder and Feldmesser (2). The nematodes on the filter are washed with water. The filter is then removed from the suction flask and the nematodes are flushed off with a wash bottle into a Syracuse watch glass where they are left undisturbed for at least 1 hour. Counts are then made on the number of inactivated worms and the total number of worms in four low-power fields (20x) of a stereomicroscope. The under sides of the watch glasses are quartered with a wax pencil to assure observation of four different fields. Percent of total inactivation is determined and the compounds rated as in contact test A.

EXPERIMENTAL RESULTS

During 1957, comparative tests were made of ten nematocides, using Contact Tests A and B concurrently. Chemicals were tested at 1000 ppm concentration, using three replicates, by each test method. Nematodes for each comparative test were obtained from the same stock suspension and the diluted chemical was obtained from the same stock preparation. Four chemical formulations and a water control were tested in each assay. Tap water was used as a diluent or washing agent throughout these tests. Individual nematodes from the Panagrellus redivivus culture became firmly attached to the glass surface when placed in distilled water, even in specially cleaned glassware. Motility decreased, and the nematode became attached to the glass along the entire body length or at the head or tail end. This did not occur in tap water or in distilled water containing low concentrations of detergents. The results obtained in these comparative tests are shown in Table 1.

DISCUSSION

Although both tests involved about the same amount of time per tested compound, contact test B eliminated the need of handling individual nematodes. Test B required the counting of many more living and dead nematodes, as well as a check count on the dilution requirements for the stock nematode suspension. In these experiments the counting dishes yielded 20 to 40 nematodes per field which was considered a reasonable counting sample. It was necessary to stimulate immobile nematodes in test A by probing with a needle, especially for readings during the first 4 hours. With some compounds, over 50 percent became immobile but reacted on stimulation. Stimulation was considered unnecessary in test B. In test B, immobile nematodes failed to react to probing and were obviously dead; only rarely was an immobile but not totally inactivated nematode encountered.

The primary advantage of contact test B is the inclusion of the various stages of growth. The effect of the chemical can be evaluated on eggs, second-stage (newly hatched) larvae, juveniles, and adults. That selective killing is possible was demonstrated with chloropicrin, which killed gravid females within a few hours. However, after 24 hours larvae from eggs hatched in the female uterus were swimming actively in the body cavity of the dead mother. Eggs laid before the female died or eggs in the mixed population suspension hatched normally and larvae appeared unaffected by chloropicrin. Minute larvae that appeared in counting dishes of contact test B were designated as newly hatched and were not included in the counts from which the percentage figures given in Table 1 were calculated.

Inactivation times obtained for the chemicals tested were almost identical by both methods. However, limited data from a repeat test with chloropicrin indicated that a compound may not give identical ratings each time it is tested, by either method. Replicated treatments in contact tests A and B showed considerable variation in kill within the replications. With chloropicrin, for example, it was possible to get 100 percent total inactivation in one dish in 1 1/2 hours in contact test A, but not until 4 hours in the replicates. This variation was generally true with both test methods for most of the compounds. Numbers of nematodes added to tubes

Table 1. Nematocidal activity of chemicals evaluated by two contact test methods.[a]

Chemical	Contact Test Method[b]	Percent total inactivation after:[c] ½ Hr.	1½Hrs.	4 Hrs.	25 Hrs.
Test,Series 1:					
Chloropicrin	A	23	73	100	100
	B	12	77	90	100
N-caprylic acid	A	100	100	100	100
	B	100	100	100[d]	100[d]
M-nitrobenzoic acid	A	00	0	13	100
	B	15	24	35	96
Heptanoic acid	A	100	100	100	100
	B	93	100	100[d]	100[d]
Water (control)	A	0	0	0	0
	B	4	7	4	16
Test Series 2:					
Hexylresorcinol	A	100	100	100	100
	B	100	100	100	100
Potassium permanganate	A	23	40	87	100
	B	4	77	97	100[d]
Trans-cinnamic acid	A	0	10	100	100
	B	69	49	96	100
Water (control)	A	0	0	0	0
	B	2	3	2	27
Test Series 3:					
Chloropicrin	A	80	93	100	100
	B	86	100	100	100
N,N'-Dimethyl dithio oxamide	A	0	0	0	100
	B	9	7	16	100
1-chloro-4-iodobenzene	A	50	67	100	100
	B	52	81	100	100
Azaleic acid	A	83	100	100	100
	B	96	100	100	100
Water (control)	A	0	0	0	0
	B	3	4	3	16

[a]All chemicals tested at 1000 ppm active ingredient.

[b]Contact test method A = as described by Tarjan and Cheo (7).
Contact test method B = the aeration-agitation method described in this report

[c]$\frac{\text{Number of worms totally inactivated}}{\text{Total number of worms}} \times 100$; readings given are averages of 3 replicates.

[d]All worms broken into segments or partially disintegrated.

in contact test B were also variable, although aliquots of nematode suspensions were taken from one source. There were no deaths in controls of contact test A over the 24-hour period. The controls in test B had 2 to 7 percent deaths over the first 4 hours and this increased at the 24-hour reading. These deaths were of old nematodes. Suspensions used for contact test B showed 0 to 1 percent dead nematodes prior to use in the tests.

This study suggests that either contact test, A or B, may be used successfully in primary screening for testing nematocides and that results may be expected to be similar with both test methods. Because of the variability found in results within either method, however, it is suggested that replicated treatments be used in tests and that the averages of replicates be utilized to rate the effectiveness of the compounds.

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DEPARTMENT OF PLANT PATHOLOGY-ENTOMOLOGY,
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A SIMPLE METHOD FOR MAKING RAPID ROUTINE PHOTOGRAPHS OF NEMATODES

Virginia R. Ferris and J. M. Ferris[1]

For 2 years the authors have been using a technique to photograph nematodes that utilizes only the compound microscope without a camera. The method, probably the simplest form of photomicrography, was suggested to them by Mr. H. H. Lyon, Photographer, Department of Plant Pathology, Cornell University, Ithaca, New York. It has proved very useful for routine use with nematodes.

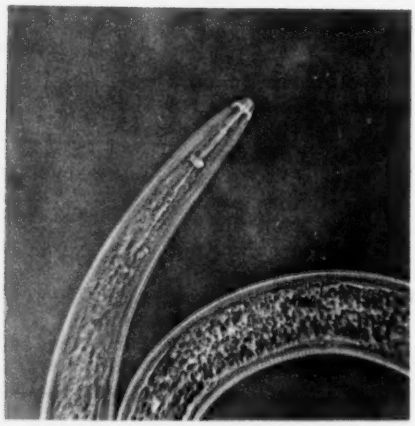


FIGURE 1. Head of Helicotylenchus sp.

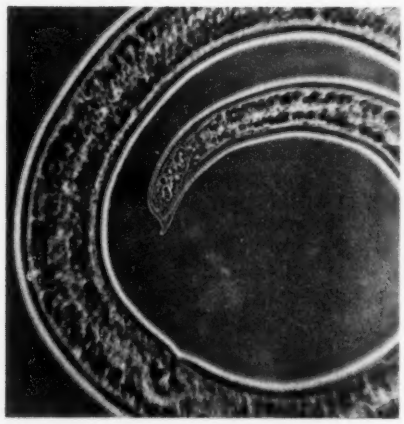


FIGURE 2. Tail of same specimen as that of Figure 1.

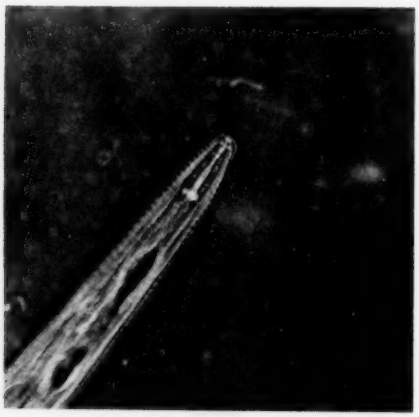


FIGURE 3. Head of Tylenchorhynchus sp.

FIGURE 4. Tail of same species as that of Figure 3.

[1]Contract Plant Pathologist, 2237 Delaware Drive, West Lafayette, Indiana; and Assistant Professor, Department of Entomology, Purdue University.

Briefly the method is as follows: The fixed (or at least relaxed) nematode, mounted on a slide, is placed on the stage of a monocular compound microscope in a darkened room. A ring stand beside the microscope with the ring placed 10 inches above the ocular lens of the microscope holds photographic enlarging paper of medium to hard contrast. When a microscope lamp that has been previously aligned and focused is turned on for a few seconds, the image of the nematode (which has been prefocused) strikes the paper. The picture is developed by the usual photographic procedures. The prints are actually negatives, but this feature has not detracted from their usefulness as records.

It is desirable to have a lamp with both a condenser system and a diaphragm so that the light can be critically focused and extraneous light cut out. Focusing of the nematode can be done on ground glass supported on the ring stand, or on onion skin paper supported by a thin piece of glass. The photographic paper can be held between two pieces of clear glass. The authors have found a discarded 5 inch X 7 inch film holder particularly satisfactory for holding the paper. The holder keeps the paper flat without the necessity of a piece of glass between the paper and the light rays.

Magnifications can be recorded by making a separate picture of the rulings on a stage micrometer immediately before or after photographing the nematode, or a calibrated ocular micrometer can be placed in the microscope and the image of its rulings photographed superimposed on that of the nematode.

The greatest number of nematode pictures can be prepared in a given time if the lenses, light focus, and filters are not frequently altered during a session. Usually an arrangement satisfactory for most specimens can be found. The exposure time for this arrangement experimentally determined for one specimen is usually satisfactory for all specimens. Use of a safe light in the room makes it possible to overlap many of the operations. The authors, working together, often expose and develop 15 to 20 nematode pictures during a period of 3 hours. The photographs, of which Figures 1 to 4 are typical, are filed along with slides, measurements, drawings and other information.

LAFAYETTE, INDIANA

BRIEF NOTES ON PLANT DISEASES

HOW GROWERS CAN DETERMINE THEIR OWN NEMATODE PROBLEMS

By P. M. Miller

A method using fumigation of soil samples in flats has been devised by which the grower himself can determine prior to planting which of his fields need fumigation.

Enough soil to fill two flats is taken from various parts of the field, thoroughly mixed, and divided into two parts: one part to be treated and the other part to be left untreated. Flats should be at least 4 inches deep and hold about 1 cubic foot of soil. The untreated soil is placed in one flat. Half of the soil to be treated is placed in another flat and the nematocide spread evenly over the surface. The nematocide may be either 10 ml of water containing 0.22 ml of a 50 percent emulsifiable concentrate of 1,2-dibromo-3-chloropropane (sold as Nemagon or Fumazone and referred to hereafter as DCP) or 3.3 g of a dry 10 percent formulation of DCP. The soil and DCP are mixed together and the remainder of the soil added to the flat.

Plants or seeds may be placed immediately in the flats on 4-inch centers. Tomatoes, okra, or cucurbits make good general test plants, or the grower may use the plant which will be grown in the field from which the soil was taken. Tobacco, onions, and garlic may be injured by DCP and cannot be used. Cuttings may be used if woody plants are to be put into the field.

Vigor and root appearance is determined 8 weeks after planting but more time may be allowed for slower growing woody plants. More vigorous plants and roots in treated flats indicate that a nematode problem exists where the soil was taken.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CONNECTICUT

ARE VIRUSES CONCERNED IN PEAR DECLINE?

By J. R. Kienholz[1]

Evidence is accumulating to indicate that Oriental rootstocks are implicated in the pear decline[2,3] occurring in the Pacific Northwest. Why some trees collapse with a "quick decline" whereas others degenerate over a longer period of time has not been satisfactorily explained.

Since 1947 many species and varieties of pears have been topworked on a tree infected with the stony pit virus at Hood River, Oregon, to record the symptoms produced by this virus on various pear selections. This tree consists of a French seedling rootstock with an intermediate trunk stock of Vicar of Wakefield and Anjou as the commercial top variety. Several other virus diseases, besides stony pit, are now recognizable in this specimen tree. These include vein yellows, ring pattern, red mottle, and sooty ring spot[4].

It is of considerable interest that of over 60 varieties and species of pears growing on this tree, only Pyrus pyrifolia (P. serotina) and P. betulaefolia have shown an incompatible reaction. P. betulaefolia was budded into this tree August 13, 1954. The resulting shoot had grown more than 18 inches in length when it suddenly died during July 1957. The collapse was similar to the quick decline observed in commercial pear orchards. P. pyrifolia was also budded into this tree in 1953. By 1958 a shoot over 4 feet long with several side branches which bore fruit during 2 years had developed. During June 1957 the leaves on this branch suddenly became necrotic and dropped, but the buds remained alive. New growth developed during 1958, but defoliation again occurred in June, and it appears practically certain this limb will die within another year or two. This performance is somewhat analagous to the behavior of "slow decline" in commercial pear orchards. Unfortunately only a few of the Oriental species that are sometimes used as pear rootstocks have been grown on this specimen tree.

True pear decline has not been observed in Hood River pear orchards, probably because Oriental rootstocks have rarely been used in this district. The observations reported here suggest that if certain viruses are present in trees of Oriental and French combinations, pear decline symptoms which have been excited by other factors may be hastened or aggravated. Since incompatibility occurs when Oriental species are grown as either the top or the root with P. communis, it may be helpful to consider this fact in planning future tests.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, HOOD RIVER, OREGON

[1] J. R. Kienholz, Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Hood River, Oregon.

[2] Woodbridge, C. G. 1957. What we know about pear decline. Washington State Hort. Assoc. Proc. 53: 49-54.

[3] Anon. 1958. Jap roots are blamed as pear decline cause. Better Fruit 54: 15.

[4] Posnette, A. F. 1957. Virus diseases of pears in England. Hort. Science 32: 53-61.

BOOK REVIEW

INTERNATIONAL CODE OF NOMENCLATURE OF BACTERIA AND VIRUSES. Edited by the Editorial Board of the International Committee on Bacteriological Nomenclature. XXII + 186 pages. June 1958. Iowa State College Press, Ames, Iowa. $3.50.

Bacteria have presented much the same nomenclatorial problems as have confronted botanists and zoologists concerned with other groups of organisms. Tentatively they have been covered by the Botanical Code, although it was early recognized that special provisions were necessary to care for them satisfactorily. This need led bacteriologists, with the cooperation of the International Botanical Congresses, to undertake the preparation of an adequate code of bacterial nomenclature.

A tentative code was issued in 1948 (Journal of Bacteriology) and 1949 (Journal of General Microbiology). The present revised edition represents the work of the International Committee on Bacteriological Nomenclature of the International Association of Microbiological Societies. It embodies a complete reworking of the original edition based on the proposals received from many interested bacteriologists and the decisions of the judicial commission. A detailed history of the development of the Code forms the "Foreword" to the present edition.

The Code itself is presented in four chapters, (1) general considerations covering scope of the code and related matters, (2) the principles recognized, (3) rules and recommendations, and (4) provisions for modification and amendment of the rules.

Appendices include opinions on the nomenclature of the bacteria as rendered to date by the judicial commission and a series of tables listing conserved and rejected names.

Annotations under each rule provide concrete examples and give reasons for adopting the rule in the form used, noting significant resemblances and differences between it and the pertinent rule in the Botanical and Zoological counterparts.

The Code follows the botanical rules very closely. There are a few points of divergence such as the provision that new names of taxa of bacteria are excepted from the general requirement of a Latin diagnosis. In strong contrast to the botanical code, the principle of conserving specific epithets is accepted and a number have already been approved.

As the title implies this code of nomenclature is intended to apply to bacteria and related organisms and the viruses. However, with the exception of one rejected generic name there are no specific references to viruses in the present text, nor are there any present indications that the virologists will accept the code as a basis for virus nomenclature.

A great deal of thought and an immense amount of hard work have gone into the preparation of this Code. It is presented in an attractive and very usable format and provides an excellent basis for permanent stabilization of bacteriological nomenclature. -- JOHN A. STEVENSON

CORRECTION

REPORTER, September issue (Volume 42, Number 9): Page 1030, in first paragraph of main text, third sentence, read C. J. Olson, instead of C. F. Olson. Page 1032, second paragraph, line 5, insert one of before 25 tobacco plants. The sentence should read "In a soil-transmission test made in parallel with the Market Orange virus described in the preceding section one of 25 tobacco plants developed etch symptoms in leaves from exposure to root infection."

ANNOUNCEMENTS

TAPE RECORDINGS OF THE SPECIAL ADDRESSES AND SYMPOSIA, AMERICAN PHYTOPATHOLOGICAL SOCIETY'S GOLDEN ANNIVERSARY MEETING: The special addresses and symposia, with discussions, given at the 50th Anniversary Meeting at Bloomington, Indiana, were recorded on magnetic tape. The recordings were made single-track at a speed of 7 1/2 inches per second on professional model equipment so they have full fidelity and are complete.

Copies of any part of these programs, re-recorded double-track at 3 3/4 inches per second, may be obtained at cost from Audio Visual Center, Purdue University, upon special order. Orders will be accumulated until October 15, 1958, then the copies will be made and mailed promptly. The tape and reels for each copy will be furnished at cost and a labor charge of $1 per hour of recording time plus postage will be made.

Since the symposia were all 3 1/2 to 4 hours in length, the cost of 1 copy of each will be about $8.00 if ordered singly. There may be a slight saving on tape costs if more than one symposium is ordered. The cost of individual papers (with discussions) of the symposia, will be approximately $2.00, depending upon length.

The special addresses by Stakman, Stevenson, Walker, McNew, Harrar, and Horsfall, complete with introductions, will each cost from $2.00 to $3.00.

Please send orders (without money, payment to be made later), to:

Audio-Visual Center,
Memorial Center,
Purdue University,
West Lafayette, Indiana

Attn: Ron Baker

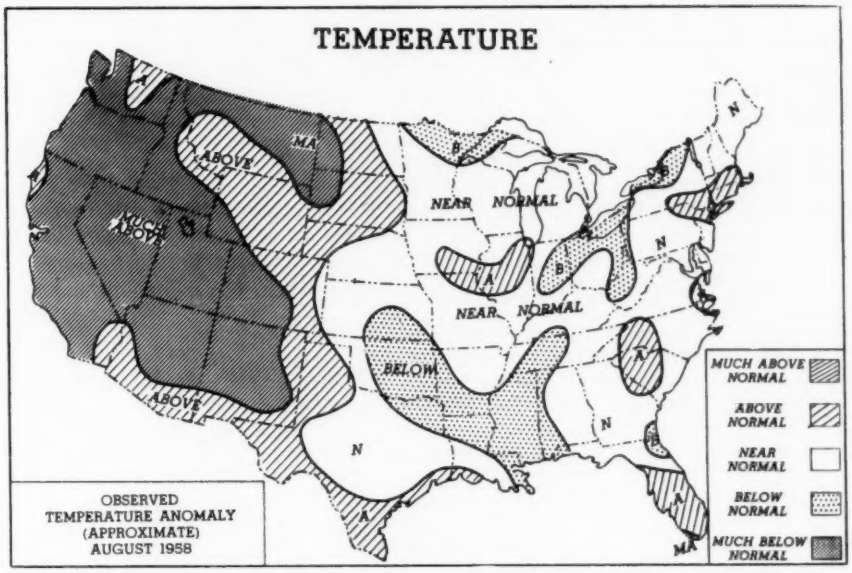
PHOTOGRAPHS OF INDIVIDUAL EXHIBIT BOOTHS, AMERICAN PHYTOPATHOLOGICAL SOCIETY'S GOLDEN ANNIVERSARY MEETING: Mr. Howard H. Lyon, Photographer for the Cornell Department of Plant Pathology, Ithaca, New York, secured photographs of all the individual booths at the Anniversary Exhibit at Bloomington.

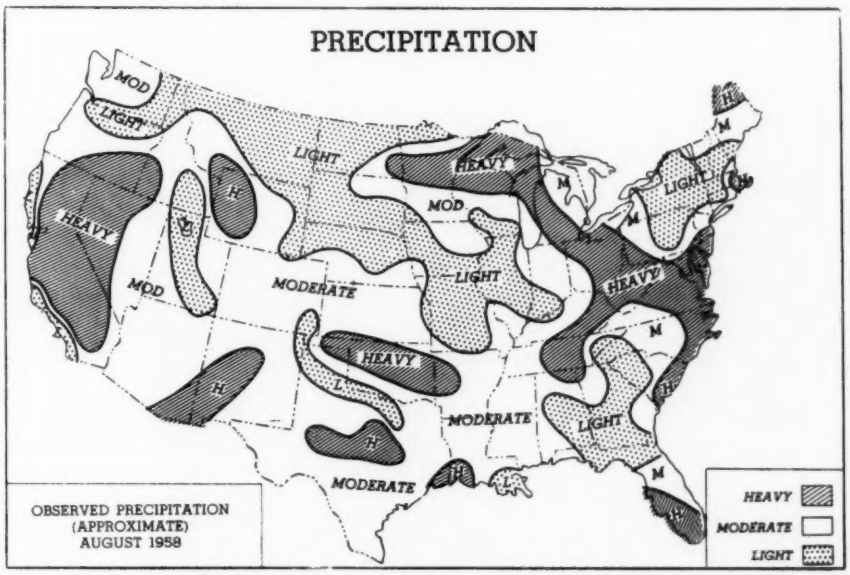
A complete set of 23 Kodachrome transparencies is available at $15.00 per set, or individual slides at $1.00 per slide.

Also available are 8 x 10 black and white prints, at $12.00 per set of 25 different prints, or $1.00 per individual print.

Orders and checks should be sent to Mr. Lyon personally before the deadline of November 30, after which he will not be able to fill orders. At this cost price, it is necessary that Mr. Lyon prepare all prints and mail them at one time; also it will take an additional 4 weeks to obtain the duplicate Kodachromes.

When ordering individual pictures, be sure to specify the name of the exhibit.





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.